An assessment of the potential of edible insect consumption in reducing human nutritional deficiencies in South Africa while considering food and nutrition security aspects.

by

Anja Lategan

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Supervisor:

Prof. G.O. Sigge

Co-supervisor:

Ms. M. L. Marais

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Abstract

Between 2012 and 2014, more than 2 000 new cases of severe malnutrition in South Africa have been reported. Staple food products are viewed as having insufficient micronutrient contents and limiting amino acids (lysine, tryptophan and threonine). Therefore, in following a monotonous diet of maize and wheat products, the risk of micronutrient deficiencies increases. Even after mandatory fortification of staple food products in South Africa in 2003, high levels of micronutrient deficiencies still exist. In this research assignment, the potential of edible insects frequently consumed in South Africa, in ameliorating South Africa's most prevalent nutrient deficiencies (iron, zinc, folate, vitamin A and iodine) was assessed. The primary data collection method consisted of searching databases and identifying and critically assessing existing literature.

The majority of edible insects contained favourable nutrient contents, except for iodine, vitamin A and tryptophan, which were limited. The katydid (*Ruspolia differens*), jewel beetle (*Sternocera orissa*), African thief ant (*Carebara vidua*) and mopane worm (*Gonimbrasia belina*) were identified as insects containing significant amounts of micronutrients. The adult *Ruspolia differens* had the highest iron content (117.2 mg.100g^{-1} product), more than brown bread flour (2.5 mg.100^{-1} product). The adult of *Sternocera orissa* provides half of the RDA of zinc when consuming 10.6 g product. Consuming 58.7 g *Carebara vidua* in the adult phase, will result in 50% of the RDA of folic acid being met. *Ruspolia differens* and *Gonimbrasia belina* were also identified as having favourable lysine, tryptophan and threonine contents. *Ruspolia differens* and *Gonimbrasia belina* contain 91.3 mg and 44.4 mg lysine per gram protein. *Gonimbrasia belina* larvae further contains a tryptophan content of 29.6 mg g^{-1} protein, whereas favourable a threonine content has been established in *Ruspolia differens* (53.3 mg.g^{-1} protein).

The Kjeldahl method was still the preferred method for protein determination of edible insects. Due to the limited amount of alternative methods utilised, no conclusions were made on whether the Kjeldahl methods leads to an overestimation of protein or if amino acid analysis provides more reliable results. Furthermore, other external factors, including geographical area, processing method, chitin content and Kp adjustment, also affects edible insects' protein content.

An increase of 33% in the edible insect market is projected between 2018 – 2022 when compared to every US$1 billion in the global meat market. This is still miniscule compared to the global meat market. Standardising food safety systems and incorporating insects into well-known products, have been proposed as promoters for edible insect market growth. Whole termites were the most expensive protein source when compared to chicken breast fillets, French polony, beef mince and chicken livers. This results in excluding a majority of the population, who resides in urban areas and does not have access to harvesting sites.

This research assignment met the objectives in accentuating the favourable nutrient contents of edible insects and the potential to assist in reducing South Africa's most prevalent nutrient

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deficiencies. Concerns and gaps however exist, but this assignment provides the platform for future research to focus on conducting studies in South Africa to determine the nutritional content of edible insects, standardise external factors, and to determine the protein content through various methods. Edible insects in South Africa has endless potential in alleviating the food insecurity.

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Opsomming

Tussen 2012 and 2014, is daar meer as 2 000 nuwe gevalle van ernstige wanvoeding in Suid-Afrika gerapporteer. Stapelvoedselprodukte word beskou as onvoldoende in mikronutriëntinhoud en beperkende aminosure (insluitend lisien, triptofaan en treonien). 'n Beperkte dieet van mielie- en koringprodukte verhoog dus die risiko van mikronutriënt tekorte. Selfs nadat die verpligte fortifisering van stapelvoedselprodukte in Suid-Afrika ingestel is in 2003, word daar steeds hoë vlakke van mikronutriënt tekorte gevind. In hierdie navorsingsopdrag is eetbare insekte wat gereeld in Suid-Afrika verbruik word, se potensiaal om ‘n verbetering aan te bring in Suid-Afrika se mees algemene nutriënttekorte (yster, sink, folaat, vitamien A en jodium), ondersoek. Databasis soektogte om bestaande literatuur te identifisieer en krities te assesseer was die primêre data insamelingsmetode.

Die oorgrote meerderheid van eetbare insekte het ‘n gunstige nutriëntinhoud, behalwe vir ‘n beperkte vlakke van jodium, vitamien A en triptofaan. Die katydid (*Ruspolia differens*), kewer (*Sternocera orissa*), mier (*Carebara vidua*) en mopanie wurm (*Gonimbrasia belina*) is geïdentifiseer as insekte met ‘n beduidende mikronutriëntinhoud. Die volwasse *Ruspolia differens* het die hoogste ysterinhoud (117.2 mg.100g^{-1} produk), meer as bruinbroodmeel (2.5 mg.100^{-1} produk). Die volwasse *Sternocera orissa* verskaf die helfte van die aanbevolle daaglikse toelating (ADT) vir sink wanneer 10.6 g produk ingeneem word. Die inname van 58.7 g *Carebara vidua* in die volwasse fase sal 50% van die ADT vir foliensuur verskaf. *Ruspolia differens* en *Gonimbrasia belina* is ook geïdentifiseer met ‘n gunstige lisien-, triptofaan- en treonieninhoud. *Ruspolia differens* en *Gonimbrasia belina* bevat 91.3 mg en 44.4 mg lisien per gram proteïene. Die *Gonimbrasia belina* larva het verder 'n triptofaaninhoud van 29.6 mg g^{-1} proteïene, terwyl 'n gunstige treonieninhoud in *Ruspolia differens* (53.3 mg.g^{-1} proteïen) vasgestel is.

Die Kjeldahl metode word nogsteeds verkies om die proteïeninhoud van eetbare insekte te bepaal. Vanweë die beperkte hoeveelheid alternatiewe metodes wat gebruik was, kon geen gevolgtrekkings gemaak word of Kjeldahl metodes tot 'n oorskatting van proteïeninhoud lei, en, of aminosuuranalise meer betroubare resulate lewer nie. Verder, eksterne faktore soos byvoorbeeld geografiese area, prosesseringsmetode, chitieninhoud en Kp-aanpassing, beïnvloed ook eetbare insekte se proteïeninhoud.

'n Aansienlike toename van 33% in die eetbare insektemark word tussen 2018 en 2022 verwag in vergelyking met elke US$ 1 miljard in die globale vleismark. Dit is egter steeds gering in vergelyking met die globale vleismark. Standaardisering van voedselveiligheidstelsels en byvoeging van insekte in bekende produkte, word voorgestel om die aanvraag vir eetbare insekte te laat toeneem. Heel termiete is ‘n duurder proteïenbron in vergelyking met hoenderborsfilette, Franse polonie, gemaalde beesvleis en hoenderlewers. Dit lei daartoe dat 'n groot hoeveelheid van die bevolking uitgeskakel word, veral diegene wie woonagtig is in stede en nie toegang het tot versamelingsareas nie.

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Hierdie navorsingsopdrag het die doelwitte bereik om die gunstige nutriëntinhoud van eetbare insekte te beklemtoon en het die potensiaal om Suid-Afrika se mees prominente voedingstoftekorte te help verminder. Bekommernisse en gapings bestaan egter steeds, maar hierdie opdrag bied die platform vir toekomstige navorsing. Verdere studies kan spesifiek daarop fokus om die voedingswaarde van eetbare insekte in Suid-Afrika te bepaal, eksterne faktore te standaardiseer en verskillende metodes te gebruik om die proteïeninhoud te bepaal. Eetbare insekte in Suid-Afrika beskik oor eindelose potensiaal om die voedselsekuriteit te verbeter.

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List of Abbreviations

AA: Amino Acid

ADT: Aanbevolle Daaglike Toelating

BCE: before the Current Era

CNS: Carbon, Nitrogen and Sulphur

FAO: Food and Agricultural Organisation

GHG: Greenhouse Gas

HACCP: Hazard Analysis Critical Control Points

Kp: Protein-to-nitrogen conversion factor

LMIC: Low-to- Middle-Income Country

MRC: Medical Research Council

NAMC: National Agricultural Marketing Council

NFCS-FB: National Food Consumption Survey – Fortification Baseline

PRISMA: Preferred Reporting Items

RAE: Retinol Activity Equivalent

RBC: Red Blood Cell

RDA: Recommended Dietary Allowance

RDI: Reference Dietary Intakes

SAFOODS: South African Food Data System

SADHS: South African Demographic and Health Survey

SANHANES-1: The South African National Health and Nutrition Examination Survey

SSA: Sub-Saharan Africa

UNICEF: United Nations Children's Fund

WHO: World Health Organisation

ZAR: South African Rand

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Chapter 1: Introduction

The international food crisis in the 1970's, resulting in large food price increases, is often viewed as the starting point of the food security revolution (Maxwell, 1996). The Green Revolution was the major driving force behind strategies which promoted food production intensification (De Schutter & Vanloqueren, 2011). Food security consists of four integral concepts, namely "availability", "accessibility", "utilisation" and "adequacy" (Barrett, 2010). The Green Revolution, however, primarily focused on the "availability" domain. Amartya Sen, Nobel Prize winner in 1998 for his work on welfare economics, later opposed the Green Revolution strategy and accentuated the importance of "accessibility" to ensure food security (De Schutter & Vanloqueren, 2011). Research indicated that even though mass production increased the mouths that were fed, it did not result in eradication of malnutrition (Maxwell, 1996). Increasing food production will be in vain if consumers cannot access these food products (Meenar & Hoover, 2012).

Viewing the Green Revolution approach, it is evident that the process was intrinsically flawed. Not only was mass production unable to achieve food security but resulted in the exploitation of natural resources (De Schutter & Vanloqueren, 2011). In assessing the past food production approaches, valuable information can be obtained to effectively plan for future production.

In the 21st century, global food and nutrition outcomes are dampened due to continuous constraints, including the ever-growing population (predicted to exceed 9 billion by 2050), increasing demand for animal-based products, prevalence of malnutrition, depletion of non-renewable resources and rise of global warming (Godfray *et al*., 2010). Additionally, by 2050, double the amount of food needs to be produced to meet global consumer demands (Tomlinson, 2013).

Lang (2009) added that diets in developed countries often consist of large volumes of highly processed animal-based food products. Not only is this diet associated with an increased risk of non-communicable diseases but places an enormous amount of pressure on the earth's resources (Godfray *et al*., 2010). The production of fertilizer intended for the growth of livestock feed and the amount of livestock faecal waste generated through farming, results in 65% of the total N_2O released. Furthermore, the production of 1 kg of beef results in 14.8 kg of CO_2 emitted (Van Huis, 2013). It is therefore no coincidence that through assessing the enormity of greenhouse gas (GHG) emissions, livestock production has been directly associated with the acceleration of global warming (Capper, 2013).

The anticipated price increase of animal-based food products will force especially low-income groups, to resort to cheaper alternatives including maize and wheat products (Gerbens-Leenes *et al*., 2010; Schönfeldt & Hall, 2012; Van Huis, 2013). Following a monotonous diet however often restricts the quality and quantity of nutrients consumed. It is therefore not uncommon that a monotonous diet has been associated with a high prevalence of nutrient deficiencies (Awika, 2011). Current available statistics are alarming. Vitamin A deficiency is responsible for over a million child

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deaths per annum while 29.8% of the global population have insufficient iodine intakes (Adamson, n.d.; Andersson *et al*., 2012). Further, more than 50% of all pregnant women worldwide are anaemic (Chadha & Oluoch, 2003). In South Africa alone, more than 2 000 new cases of severe acute malnutrition have been reported between 2012 and 2014 (McLaren *et al*., 2017).

Through considering the above mentioned, a dire need for a sustainable food source to decrease the prevalence of deficiencies by either serving as an alternative food source or as a supplement to a staple diet, while adhering to food safety protocols and consumer preferences exists. Entomophagy, the consumption of insects, has received ample attention in the past few years as a potential alternative or supplementary food source (Van Huis *et al*., 2015). Insects have however been part of human diets for centuries. Cave paintings dating back to between 3 000 and 9 000 BCE in Spain and Mexico, illustrates the utilisation of insects as food source (Akhtar & Isman, 2018).

Compared to livestock production, insects have a favourable nutritional content (Ramos-Elorduy, 2009). The mopane worm (*Gonimbrasia belina*), contains all essential amino acids and significant amounts of iron and zinc (Bukkens, 2005). Netshifhefhe *et al.* (2018) further indicates that edible termites of South Africa are a good source of nutrients. Insect rearing also has a considerably lower environmental impact than livestock production (Premalatha *et al*., 2011). A study done by Oonincx *et al*. (2010) illustrated that CO_2 and NH_3 emissions in five insect species were significantly lower per kg of metabolic weight than when compared to cattle and pigs.

The majority of western countries do however currently not acknowledge insects as a substantial food source (Deroy *et al*., 2015; Ng'ang'a *et al*., 2018). Consumer acceptance of insects is often rejected due to neophobia or the "disgust" factor (La Barbera *et al*., 2018; Schlup & Brunner, 2018). The incorporation of insects into existing products has been proposed as a possible solution to increase consumer acceptance (Hartmann *et al*., 2015). Other concerns regarding edible insects include allergenic reactions, microbiological and chemical hazards, market accessibility and viability as a food source (Rumpold & Schlüter, 2013; Dzerefos *et al*., 2014).

The main objective of this research project is to assess the potential of edible insects frequently consumed in South Africa, in ameliorating South Africa's most prevalent nutrient deficiencies. During the primary sub-objective, parallels are drawn between the nutrients devoid in South African staple food products and frequently consumed insects rich in the specific nutrients. The secondary sub-objectives includes the investigation of the viability of edible insects as alternative or supplementary food source to the South African diet. Factors that will be assessed include, consumer acceptance, sustainable production and market potential, growth and opportunities compared to other food sources. This research project will serve as a starting point for future research on how frequently consumed edible insects in South Africa, can be utilised as potential supplementary food sources in conjunction with staple food products. Critical issues will be identified through in-depth research on edible insects in South Africa and recommendations will be made.

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Chapter 2: Literature Review

2.1 Introduction

“Insects create the biological foundation for all terrestrial ecosystems. They cycle nutrients, pollinate plants, disperse seeds, maintain soil structure and fertility, control populations of other organisms, and provide a major food source for other taxa.” (Scudder, 2017). This quote emphasises the indispensability of insects and the role they play on all levels of the ecosystem.

By 2050, it is predicted that the world would have to accommodate 2.4 billion more people than in 2013 (UN News, 2013). Meeting the nutritional demands of the increasing population in an environmentally sustainable manner, has proven an arduous task. It is reasonable to expect that as the global population increases, the demand for food products, especially animal-based products, will increase accordingly (Megido *et al*., 2016). Overexploitation of renewable and non-renewable resources, greenhouse gas emissions (GHG) and waste generation are all consequences of livestock mass production (Pelletier & Tyedmers, 2010). It is therefore evident that upscaling of livestock production is not a sustainable option (Capper, 2013). Insect production on the other hand requires less resources, feed, land and water compared to livestock breeding (Kouřimská & Adámková, 2016).

Furthermore, in the 21st century, nutrient deficiencies are still acknowledged as a universal problem. A lack in the variety of food products consumed, due to various social, economic and political factors, contributes to the level of deficiencies present (Bailey *et al*., 2015). Bukkens (1997) concluded that due to the favourable nutrient content of insects, the potential exists in acting as supplementary food source alongside grain products.

Just as wide variety of commonly consumed food sources which poses risks such as allergic or high heavy metal contents, the same is evident for novel product or ingredient such as insects. The distinction between novel and commonly consumed products regarding ingestion concerns can however be made on the basis of knowledge or information available for the specific product. In the case of novel products, a lack in global standardisation and implementation of regulations can exacerbate feelings of uncertainty (EFSA Scientific Committee, 2015). Consideration must be given to the phylogenetic differences present between insects and other forms of livestock and influence it can have on the specific risks associated with the food product (Belluco *et al*., 2015).

Feng *et al*. (2017) highlighted incidences of allergenic reactions in China after the ingestion of cicadas and silkworm pupae. Other concerns include heavy metals, such as lead present in grasshoppers in Mexico and insects acting as carriers of foodborne pathogens (Handley *et al*., 2007; Belluco *et al*., 2013).

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2.2 Prevalence of nutrient deficiencies

Undernutrition and micronutrient deficiencies are two forms of malnutrition that constantly strains global health outcomes (Tao, 2018). More than 2 billion people worldwide has been affected by nutrient deficiencies (Bailey *et al*., 2015). Factors contributing to malnutrition include nutrient absorption barriers, lack of knowledge regarding nutrition, poor diet choices and poverty (Schroeder, 2008; Bailey *et al*., 2015).

The most prevalent micronutrient deficiencies (iron, vitamin A, iodine, folate, zinc) on a global scale, are also the highest occurring micronutrient deficiencies in South Africa (Ramakrishnan, 2002; Wenhold & Faber, 2008). Micronutrient supplementation plays an important role in reducing deficiencies, but according to UNICEF (2010) the availability and distribution of these supplements are often problematic.

Bailey *et al*. (2015) mentioned that approximately a third of all people worldwide are suffering from iron deficiency, classifying it as the most common micronutrient deficiency. Iron deficiency anaemia contributes to 37% of all maternal deaths in Africa (Ortiz-Monasterio *et al*., 2007; Akhtar & Isman, 2018). In South Africa, 17.5% of the population are suffering from anaemia (Shisana *et al*., 2013). People suffering from iron deficiencies and anaemia often have low energy levels, which will inevitably reduce their productivity (Phatlhane *et al*., 2016).

Statistics on the prevalence of vitamin A deficiencies primarily focuses on children under five years of age and women of child bearing age (Bailey *et al*., 2015). According to the WHO (2009), it is estimated that a third of all children under five years of age are suffering from vitamin A deficiencies. In South Africa, 13.3% of females are suffering from a vitamin A deficiency (Shisana *et al*., 2013). Regarding research of vitamin A supplementation, it is however, evident that it significantly contributes to reducing the mortality rate (Micronutrient Initiative, 2009).

Iodine plays a significant role in the wellbeing of individuals. Inadequate iodine levels in the diet can increase the risk of brain damage as well as delayed mental and physical development in children (Ahmed *et al*., 2012). Globally, approximately 2 billion people are affected by iodine deficiencies (Bailey *et al*., 2015). Furthermore, 58 million people in Africa do not meet the recommended iodine intake (Andersson *et al*., 2012). The fortification of salt with iodine is viewed as the most effective manner of increasing iodine consumption and reducing the prevalence of deficiencies (Bhutta *et al*., 2013). An article by Jooste & Zimmerman (2008), indicated that South Africa has reached “optimal iodine nutritional status”. It must however be taken into consideration that the statement originated from a survey in which only the iodine status of primary school children were assessed (Immelman *et al*., 2000). The suitability of this article must therefore be considered when assessing the iodine status on a national level amongst all age groups. In addition to the aforementioned, the accuracy and reliability of the standard method employed in determining iodine status, has been questioned (Soldin, 2002). The iodine content is often determined through the

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obtainment of urinary samples which is then compared to the standard reference values (Charlton *et al*., 2018b). It is however prevalent that significant variation in the iodine content of the urinary samples can occur on a daily basis (Soldin, 2002). In considering this, the realisation was clear that an arduous task in establishing an accurate national or global iodine status was prevalent (Andersson *et al*., 2012; Charlton *et al*., 2018b).

Data regarding global folate deficiency rates are however scarce, often only done on small scale (McLean *et al*., 2008). The average folate dietary intake of women of reproductive age in South Africa is below the Recommended Dietary Allowance (RDA) of 400 µg, ranging between 82 – 334 $\mu g.day^{-1}$ (NFCS-FB, 2007; Harika *et al*., 2017; Mahan & Raymond, 2017). Folic acid is essential during pregnancy, as it decreases the risk of neural tube defects (United Nation's Children Fund, 2013). The fortification of staple food products with folate and the provision of supplementation before and during pregnancy on a national level can potentially aid in the reduction of folate deficiencies (Hoyo *et al*., 2011; Metz, 2013). Supplementation accessibility can however be identified as one of the major barriers in the reducing folate deficiencies, as it is often only obtainable at health clinics (Bhutta *et al*., 2013).

Zinc plays a crucial role in the functioning of the thyroid (Bailey *et al*., 2015). Moreover, the risk of diarrhoea can be reduced with adequate intake of zinc, and inevitably lead to increased absorption of nutrients (UNICEF, 2010). However almost a third of the global population is unable to experience these benefits as they are zinc deficient (Akhtar & Isman, 2018). In Africa, 23.9% of the total population have inadequate zinc levels (Bailey *et al*., 2015). Furthermore, certain illnesses, such as the Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS), decreases the zinc absorption rate (Sneij *et al*., 2016). Statistics South Africa (Stats SA), estimated that in 2017, approximately 10 million South Africans between 15 – 49 years of age, are HIV positive. The potential therefore exists that the HIV positive statistics can contribute to the zinc deficiency rate in this age group.

In conclusion, Van der Waals & Laker (2008) mentioned that even though the severity of micronutrient deficiencies is acknowledged as a focal problem in South Africa, statistics and data (especially of men) are insufficient in illustrating the full extent of the situation. It is therefore impossible to effectively construct and implement programs to alleviate the deficiency rates if the enormity of the problem is unknown.

2.3 Staple foods in South Africa and the impact on nutritional outcomes

2.3.1 Micronutrient content

Research has indicated that the consumption of a diverse diet, consisting in a wide variety of food products, decreases the risk of nutrient deficiencies (Chakona & Shackleton, 2017). In 2009, South African citizens on average consumed 104 kg maize, 60.9 kg wheat, 42.9 kg vegetables and 34.8

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kg fruit per annum (Ronquest-Ross, *et al*., 2015). Fruit and vegetables are a vital source of micronutrients. The reality however is that more than half of the South African population only consumes between one and three portions of fruit and vegetables a day. This is well below the World Health Organization's (WHO) recommended five portions (Shisana *et al*., 2013).

A report published by Umberger (2015) illustrated cereal product consumption statistics between various income groups. Comparing the cereal consumption rate of Asia, Latin America, North Africa and Sub-Saharan Africa (SSA), it was prevalent that the highest cereal consumption rate for each region, was in the lowest income quintile. Consuming large amounts of grain products, especially refined grain products, have been linked to various health concerns. Limiting the consumption to certain food groups increases the probability of inadequate nutrient intakes (Awika, 2011). Furthermore, research has indicated a clear link between a diet high in refined grain products, obesity and non-insulin-dependent (type II) diabetes mellitus (Gross *et al*., 2004).

Grain products are known to contain low quantities of iron, zinc, and vitamin A, which are some of the most common nutrient deficiencies worldwide (Ortiz-Monasterio *et al*., 2007). The bioavailability of iron and zinc in grain products are often compromised due to the presence of phytic acid which naturally occurs in the staple products. The decrease in bioavailability then subsequently results in a reduced absorption rate (Nuss & Tanumihardjo, 2010; Suri & Tanumihardjo, 2016). In conjunction with the reduction in zinc bioavailability, the zinc content is further decreased through the extensive milling process to produce a highly refined product. The wheat kernel's germ contains the largest concentration of zinc. However, the milling process results in the removal of the germ, leading to a massive decrease in zinc content of up to 80% (Suri & Tanumihardjo, 2016).

Various studies have indicated that the staple food products (sifted maize meal, brown bread and white bread) are not a significant source of vitamin A and were often indicated as zero before fortification (Duvenage & Schönfeldt, 2007; Van Jaarsveld *et al*. 2015). Wolmarans & Danster (2008) further mentioned that if certain nutrients are listed as zero in the SAFOODS (South African Food Data System), the food item either does not contain the specific nutrient or the low quantities deems it infeasible to determine. This can be contributed to limited fat content in grain products, consequently resulting in low quantities of vitamin A which is a fat-soluble vitamin (Dewettinck *et al*., 2008).

Iodisation of salt became compulsory in South Africa in December 1995. However, manufacturers are exempted from the compulsory utilisation of iodised salt during food production (Charlton *et al*., 2018a). A study done by Harris (2003) was one of the few studies which investigated the use of iodised salt in South Africa during the bread and bread premix manufacturing process. Conclusions of the study indicated that numerous manufacturers are often unaware of the utilisation of iodised salt during production. The possibility therefore exists that the iodine content of staple food products can be higher than anticipated.

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Despite the mandatory fortification of staple food products with vitamin A, thiamine, riboflavin, niacin, folic acid, pyridoxine, iron and zinc, since 2003, high levels of micronutrient deficiencies still exist (NFCS-FB, 2007; Motadi *et al*., 2015). A survey conducted by Yusufali *et al*. (2012) revealed that the mandatory micronutrient quantities added to staple food products at mills throughout South Africa, were not complying with the fortification legislation. This can potentially explain the perplexingly high nutrient deficiency rate. The WHO/FAO (2006), accentuated the fact that it is impossible for food fortification to singly eradicate micronutrient deficiencies. Furthermore, the role of micronutrient interactions in the absorption rate must also be acknowledged. Thurnham (2004) investigated the interactions of micronutrients with certain types of alcohol, drugs and tea intakes. The potential exists for nutrients to interfere with each other such as iron and zinc, which will ultimately affect the absorption rate of the nutrients. Certain beverages such as tea, which contains polyphenols, reduce the non-haem-iron bioavailability and therefore potentially alter the iron absorption rate (Mascitelli & Goldstein, 2011).

2.3.2 Protein and amino acid content

Grain products are classified as being insufficient in meeting the protein requirements of consumers. Amino acids (AA's) which are often described as the "building blocks of protein", can be grouped as non-essential, conditionally essential or essential (Jalkanen *et al*., 2004; Melo-Ruiz *et al*., 2015; Fombong e*t al*., 2017). The human body possesses the ability to produce conditionally essential AA's, for example phenylalanine which can be converted to tyrosine. This can however only occur if a sufficient amount of phenylalanine is consumed and the tyrosine intake is low (Litwack, 2018). Essential AA's (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, threonine and valine) are however only obtainable from the diet due the human body's inability to produce these compounds (Belluco *et al*., 2013).

In considering the amino acid profile of maize and wheat, it became prevalent that lysine and tryptophan are limiting amino acids (Bukkens, 1997; Vasal, 2000; Dewettinck *et al*., 2008; Awika, 2011). Limiting AA's can be described as essential AA's that are present in small quantities within proteins (Finke, 2013). Lysine is often described as the first limiting AA in the majority of grain products (Awika, 2011). Pellet & Ghosh (2004) has indicated that animal-based sources overall have a higher lysine content than that of grain products. However, the significant price difference between animal-based sources and grain products will inevitably affect the affordability and accessibility of these products. It is therefore not unexpected that according to Pellett & Ghosh (2004), "lysine is the amino acid for which the largest differences occur between the diets of the rich and the poor." As with the micronutrient content, the processing of staple food products often leads to the reduction in AA content (Suri & Tanumihardjo, 2016). During the milling process, the degerming of maize can result in the original tryptophan content being reduced by up to 50% (WHO, 2000).

Threonine is another AA which is often only available in limited quantities in wheat products (Vasal, 2000; Prasanna *et al*., 2001; Jiang, *et al*., 2008). This can be contributed to the low quantities

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of threonine present in the storage proteins, namely the prolamin proteins (Shewry, n.d). A lack of these compounds deems grain products as incomplete protein sources. Insufficient essential AA's in the human diet, will therefore restrict a multitude of human biological processes from functioning optimally (Belluco *et al*., 2013; Melo-Ruiz *et al*., 2015).

2.4 Rise in demand of animal-based protein sources and environmental consequences

Research has indicated that with the increase in global wealth, population growth, the improvement of living conditions and changing consumption patterns, a rise in the demand for animal protein sources becomes evident (Delgado, 2003; Godfray *et al*., 2010). A projected increase in animal protein demand of 76% between 2007 and 2050 is expected (Van Huis, 2016). In 2009, South Africans on average consumed 58.7 kg per capita of meat on an annual basis, compared to 40.3 kg consumed in 1994 (Ronquest-Ross *et al*., 2015). Even though an increase is predicted in the consumption of animal-based protein sources, the high prices of animal protein deems it unobtainable for many low-income population groups, thus depriving them of these sources (Gerbens-Leenes *et al*., 2010; Alemu *et al*., 2017a).

The environmental impact of livestock production has been heavily criticised as it requires vast amounts of resources, including land, feed and water. Approximately 15 000 – 20 000 L of water and 10 kg of feed are needed to produce 1 kg of beef (Smil, 2002; Dobermann *et al*., 2017). Upscaling production through unsustainable production practises, will however further exploit non-renewable resources and contribute to the acceleration of the process of global warming (Alemu *et al*., 2017a). There is thus a dire need for a sustainable alternative food source which simultaneously meets the nutritional requirements of consumers (Tao & Li, 2018).

2.5 Insects as a viable alternative food source

2.5.1 Edible insects on a global and South African level

Globally, it is estimated that approximately 2 111 different species of insects are consumed, with approximately 36 edible insect species indigenous to South Africa (DeFoliart, 1997; Jongema, 2017). It is highly likely that this number is an underestimation of the actual number of edible insect species present in South Africa. According to Ledger (1971), evidence suggest that termites (*Trinervitermes trinervoides*) and bees (*Apis mellifera unicolor*) were consumed by South Africans from as early as 100 000 BCE. The Pedi tribe of South Africa has been relying on insect consumption for years to assist their nutrient intake, especially during periods of food scarcity (Bodenheimer, 1951). The Mopane worm (*Gonimbrasia belina*), is an example of an insect which is consumed and exported on large scale in South Africa (Akpalu *et al*., 2009). The harvesting of these insects however needs to be controlled and monitored. Ramos-Elorduy (2006) further indicated that due to uncontrolled harvesting, the survival of approximately 40 insect species are in danger. Other insects frequently

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consumed in South Africa, include termites (*Macrotermes falciger*, *Macrotermes natalensis* and *Macrotermes michaelseni*), stinkbugs (*Encosternum delegorguei*) and moth caterpillars (*Hemijana variegata*) (Dzerefos *et al*., 2014; Netshifhefhe *et al*., 2018).

2.5.2 Nutritional composition

2.5.2.1 Micronutrient content

Various literature sources have acknowledged the vast range in nutritional content of insects between species, gender and the specific stage of development (Oonincx, 2015; Payne *et al*., 2016; Akhtar & Isman, 2018). For example, the established iron content of insect species ranges between 1.30 – 63 mg.100 g^{-1} dry product and zinc content between 0.09 – 32.54 mg.100 g^{-1} dry product (Omotoso, 2006; Pretorius & Schönfeldt, 2012; Igwe *et al*., 2012). However, even with the wide disparity in nutritional content across various species, insects are continuously recognised as a valuable source in reducing nutritional deficiencies worldwide (Belluco *et al*., 2013; Akhtar & Isman, 2018).

Furthermore, as the magnitude of research on edible insects continue, increasingly more evidence are published on the role that diet can play in the edible insects' nutritional content (Cammack & Tomberlin, 2017; Rutaro *et al*., 2018). Findings published on *Locusta migratoria* (migratory locust), indicated that the adult *L. migratoria* had a higher iron content when fed a combination of grass and wheat bran (217 mg.kg^{-1} dry weight) as opposed to when only receiving grass as feed (151 mg.kg^{-1} dry weight) (Oonincx & Van Der Poel, 2011). Liland *et al*. (2017) further illustrated this through establishing that when the black soldier fly (*Hermetia illucens*) was provided with a high iodine food source, the iodine content of the insect meal increased accordingly.

The RDA of iron (Fe) for women between 19 – 50 years of age, is established as 18 mg Fe/ day (Mahan & Raymond, 2017). The larva of the mopane worm (*Gonimbrasia belina*) contains as much as 31 mg Fe.100 g^{-1} dry product (Bukkens, 1997). The bioavailability of the iron from edible insects however needs to be considered. The presence of haemoglobin and myoglobin is a possible factor which can influence the bioavailability of iron (Roos & Van Huis, 2017). Animal-based sources have a greater haemoprotein content than most edible insects, which can potentially result in a higher bioavailability rate (Latunde-Dade *et al*., 2016; Dobermann *et al*., 2017). Kinyuru *et al*. (2015) however added that the non-heme iron biovailability in plant-based food sources can be increased when consumed with edible insects, such as termites. Possible solutions proposed for this problem, include the reduction of chitin and the addition of a vitamin C rich food source to promote the iron absorption rate. Vitamin C has the ability to convert ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), deeming it more readily absorbable in the human intestinal tract (Gabaza *et al*., 2018). Further investigation is however needed on the bioavailability of nutrients when paired with a vitamin C source and the variance in iron compounds between insects (Roos & Van Huis, 2017; Gabaza *et al*., 2018).

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Consuming 100 g of black soldier fly larva (*Hermetia illucens*) can assist in meeting the recommended iodine intake of adults as it contains as much as 26 μg.100g^{-1} dry product (Finke, 2013). It is, however, evident that a paucity occurs in the amount of studies in which the iodine content of insects are established (Oonincx, 2015). In establishing the influence of the insects' diet on the nutrient content of the insects in question, the feed can be adjusted accordingly to meet the desired nutritional content of the insects (Liland *et al*., 2017). Since iodine deficiencies continue being a health concern in South Africa, despite mandatory iodisation of salt, investigation towards edible insects as potential supplementary source of iodine is proposed.

The termite (*M. falciger*) and the mopane worm are good sources of zinc, containing 5.30 mg.100 g^{-1} dry product and 14 mg.100 g^{-1} dry product respectively (Chulu, 2015; Kouřimská & Adámková, 2016). These insects can significantly contribute to the adults' RDA of zinc established as between 8 – 11 mg per day (Mahan & Raymond, 2017).

Rumpold & Schlüter (2013) mentioned that Coleoptera and Orthoptera orders are often viewed as significant sources of folic acid. Furthermore, the consumption of 100g of dried mealworm (*Tenebrio molitor*) contributes to almost half of adults' RDA of folate (400 mcg.day^{-1}) (Nowak *et al*., 2016; Mahan & Raymond, 2017). As the case with zinc, the processing method of choice can however significantly impact the folic acid content of insects. A study published by Kinyuru *et al*. (2010) indicated that katydid (*Ruspolia differens*) samples contained 43% less folic acid than compared to the fresh samples.

The RDA for vitamin A for adults is established as between 0.7 – 0.9 mg of retinol activity equivalents (RAEs) per day (Mahan & Raymond, 2017). The *Encosternum delegorguei* has a vitamin A content of 0.23 mg.100 g^{-1} dry product and could therefore provide support in meeting adults' RDA of vitamin A (Teffo *et al*., 2007). However, various resources have stated that most insect species does not contain remarkable amounts of vitamin A (Sánchez-Muros et al., 2014; Kouřimská & Adámková; 2016). This could raise the question as to whether insects can be viewed as significant sources of vitamin A.

Considering the before mentioned, the potential of edible insects to assist micronutrient intakes in areas troubled by high deficiency rates, needs to be acknowledged and explored further (Bukkens, 1997; Banjo *et al*., 2006; Chakravorty *et al*., 2014). It can therefore be deemed beneficial in compiling a list of the edible insect species and their respective nutritional contents in countries such as South Africa (Bukkens, 1997). The possibility then arises for insect species to form part of existing food products and in such a way contribute to high intakes of nutrients (Lautenschläger *et al*., 2017). The concern however with food-to-food fortification, such as staple food products with insects, is that the same route will be followed as the mandatory addition of micronutrient premixes. If the mills and production facilities do not adhere to the fortification or enriching guidelines, the desired outcomes (eradication of micronutrient deficiencies) will not be achieved (Yusufali *et al*., 2012). On the other hand, the promoting of edible insects as supplementary food source in

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combination with staple food consumption, has the potential to assist in meeting the RDA of nutrients, but only in those willing to practice entomophagy.

2.5.2.2 Protein and amino acid content

When considering the nutritional content of edible insects, often one of the first associations is the high protein content (Belluco *et al*., 2013). Termites contain between 35 – 65% protein, caterpillars fall between the range of 50 – 60% and crickets, grasshoppers and locusts between 41 – 91% (Van Huis, 2003). Compared to the protein content of beef (40 – 75%), insects can be viewed as a significant contender in being classified as an alternative protein source (Bessa *et al*., 2017).

The potential inclusion of edible insects into staple food products has been highlighted by De Oliveira *et al*. (2017), where the cinerous cockroach (*Nauphoeta cinereal*) was incorporated into wheat bread. The cockroach-enriched wheat flour resulted in a 49.16% higher protein content when compared to normal wheat bread. Another study published by Osimani *et al*. (2018) indicated that in enriching wheat bread with cricket powder, a higher AA content including lysine, tyrosine, threonine, valine and methionine yield was evident. It is however noticeable that as with the micronutrient content of edible insect species, a wide variety in the AA content throughout the specific orders and even amongst different species can be present (Rumpold & Schlüter, 2013; Kouřimská & Adámková, 2016; Akhtar & Isman, 2018).

Bukkens (1997) indicated that in order to assess the quality of the protein content, it is more beneficial to establish and analyse the essential AA content of insects. Igbabul *et al.* (2014), stated that insects are overall a great source of lysine and threonine but are lower in methionine content. This is in correspondence with other studies which indicated that *R. differens*, *T. molitor* and *H. illucens* have lysine and tryptophan contents which can be advantageous in supplementing low levels present in grain products (DeFoliart, 1999; Van Huis, 2013; Kouřimska & Adámková, 2016).

Furthermore, the mopane worm is known for its exceptional overall AA profile which perfectly matches the human AA requirements (Payne *et al*., 2016; Pieterse, E. (PhD), 2018, Researcher and lecturer, Stellenbosch University, South Africa, personal communication, 28 March). Another insect highlighted by Lautenschläger *et al*. (2017) for the superiority in AA content, is the African moth larva (*Imbrasia epimethea*). Further consideration should however be given to the bioavailability of amino acids and the factors influencing the outcome. Amino acids are often viewed as an enhancer in nutrient bioavailability (Fairweather-Tait & Southon, 2003).The bioavailability of amino acids are further affected by the gut microbiota through adjusting the synthesisation process, catabolism and deposition of intramuscular fat (Calvani *et al*., 2015).

2.5.3 Harvesting and cultivation of insects

Oonincx (2015) indicated that edible insects are commonly reared through two primary forms of insect production, “extensive” and “intensive”. During “extensive” production, insects often face

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various challenges including weather, disease and predator threats. During “intensive” systems the environment is favourably altered. In controlling the external factors to which insects are exposed to, an improvement in quality and yield can be expected (Ali *et al*., 2011; Krengel *et al*., 2012). Furthermore, an additional advantage of captive breeding as opposed to wild harvesting, is that in standardising the insect feed, increased uniformity in the nutritional content of edible insects can be expected (Kouřimská & Adámková, 2016).

The cultivation of edible insects, such as crickets, requires less resources compared to other animal-based protein sources. Insects require approximately 0.8 kg less feed than poultry to produce 1 kg of live weight product (Collavo *et al*., 2005; Van Huis, 2010). The environmental impact of insect rearing is also less intensive as it produces as much as 2 728 g per kg product less GHG than cattle (Oonincx *et al*., 2010).

In providing the optimal environment for house flies (*Musca domestica*), as many as 2×250^{25} offspring can be produced annually (Mitsuhashi, 2010). Agriprotein, a large-scale insect producing facility in South Africa, has the capacity to produce more than 20 tonnes of fly larvae MagMeal™ (dried fly larvae) daily (AgriProtein, n.d.). Furthermore, the cost of feed can be reduced as certain insect species can be reared on biodegradable waste (Verbeke 2015; Cortes Ortiz *et al*., 2016).

Careful consideration must be given to the sustainable harvesting of edible insects when done on large scale. Thomas (2013) mentioned that the mopane worm is an example of edible insects which through intensive harvesting throughout the years, has drastically declined in numbers. The implementation of reduced harvesting periods can potentially play a role in protecting the species (Thomas, 2013). The establishment of insect farms can further aid the process of sustainable mini-livestock breeding, especially when natural population numbers are declining (Hardouin, 1995).

Harvesters often lack the necessary skills and resources to establish insect rearing facilities of scale (Yen, 2008). The reality is that the initial establishment of large-scale insect rearing facilities are often expensive. Sufficient resources, including investments, skilled personnel, feed and processing areas are needed to ensure successful insect rearing (Cortes Ortiz *et al*., 2016). Clegg (2015) included that the rapid expansion of large-scale insect rearing facilities can further endanger local harvesters’ source of income. The solution promised is the effective collaboration between local harvesters and large-scale facilities. In including and training local harvesters, their income can increase by acting as suppliers to large scale rearing facilities (Ayieko *et al*., 2016). Research further accentuated the possibility of small-scale insect rearing, which can be managed from the residents’ homes. With the adequate resources available, insect rearing can be a cheap and less intensive food source to produce (Nakamura *et al*., 2015; Berggren *et al*., 2018).

Verbeke (2015) however pointed out the gap in research regarding mass rearing of non-indigenous insects and the possible impact on the surrounding environment. Further investigation is

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needed on whether the non-indigenous species can potentially act as a pest or disrupt the natural ecosystem in the unlikely case of escaping (Van Huis & Oonincx, 2017).

Before the commencement of large-scale production, the viability of farming specific insect species must be considered (Cáceres *et al*., 2012). According to Oonincx (2015) a harvesting and processing company in Nigeria, reared *Oryctes spp.* larvae between 1980 and 1997. The process unfortunately came to an abrupt halt due to inconsistent insect yields. Intricate knowledge on the needs of the specific species in terms of temperature, environment and feed are therefore crucial to ensure sustainable production yields (Oonincx, 2015).

2.6 Food safety aspects

2.6.1 Microbiological aspects

According to the FAO: “Compared with mammals and birds, insects may pose less risk of transmitting zoonotic infections to humans, livestock and wildlife, although this topic requires further research” (Van Huis *et al*., 2013). However, an article published by Belluco *et al*. (2013), opposes the FAO’s statement in indicating that diseases caused by micro-organisms, can be transferred from insects such as flies to humans. Consideration should however be given as to how the specific insect species and the life stage of the species are identified. Certain insect species are exclusively viewed as pests that spreads disease whereas other insect species are more well-known for being consumed and poses less of a risk (Belluco *et al*., 2015). In Nigeria, various micro-organisms, such as *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus*, were isolated from the rhinoceros beetle (*Oryctes monoceros*) larvae (Banjo *et al*., 2006). Espelund & Klaveness (2014) further mentioned that for example certain fly larvaes can be potential vectors of the *Clostridium botulism* bacteria.

The inhabitation of certain environments, such as decaying matter, possible cross-contamination and the improper processing and storage, are all contributory factors which increase the microbial load (Banjo *et al*., 2006). The correct processing and preservation of insects is therefore of critical importance to ensure safe consumption. The counts of *Enterobacteriaceae*, being sensitive to heat treatment, were significantly reduced when the insects were cooked for five minutes (Belluco *et al*., 2013; Ng’ang’a *et al*., 2018). A study published by Ng’ang’a *et al*. (2018) however indicated that the heat treatment applied to *Ruspolia differens* samples did not result in a decrease in the bacterial endospore count of the insects.

Klunder *et al*. (2012) mentioned that additionally, a combination of processing treatments, known as “hurdle technology”, can be applied in reducing micro-organism counts. These processes include roasting, cooking, boiling and storing below 5°C (Klunder *et al*., 2012). The implementation of food safety systems such as the Hazard Analysis Critical Control Points (HACCP) system

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throughout the entire edible production chain, will be paramount in ensuring the safe production of quality products on large scale (House, 2018).

2.6.2 Chemical, and heavy metals

According to Yen (2008), little evidence of humans experiencing adverse reactions due to toxic chemicals associated with insects. The cadmium and copper levels present in mopane worms from the Kruger National Park however exceeded the recommended legal limits of the United Kingdom and European Union (Greenfield *et al*., 2014). Just as the insects which have been exposed to high levels of metals due to environmental pollution, it became evident that other animals such as impalas and buffalos also exhibited high levels of copper through faecal samples collected (Grobler & Swan, 1999). Other incidences have been reported where the nicarbazin amount present in fly larvae, exceeded the prohibit levels (Charlton *et al*., 2015).

2.6.3 Allergenicity

According to Van Huis *et al*. (2013), individuals sensitive to shellfish should take caution in the consumption of certain insects, as literature has indicated the possibility of allergic reactions and even the onset of life-threatening situations such as anaphylaxis. The allergic reaction can be attributed to the occurrence of tropomyosin in insects, the same protein present in shellfish (Srinroch *et al*., 2015).

In Botswana, a case of a woman who experienced an allergic reaction was reported after the ingestion of mopane worms (Okezie *et al*., 2010). A study published by Potter (2013) further included cases of South African students experiencing allergenic reactions after ingesting body fragments of the *Locusta migratoria*. Srinroch *et al.* (2015) proposed that compiling an intensive allergen database can pave the way for increased research and treatment of allergenic reactions.

2.7 Protein content of insects: Potential factors influencing results

Numerous studies have indicated that as the number of edible insects, subjected to variety of factors including geographical area, processing method and quantification method, increases in the study analysed, the greater the chances are for a wide range of values to be present (Ramos-Elorduy *et al*., 2002; Cheng & Philips, 2014; Johnston, 2014; Payne *et al*., 2016).

2.7.1 Geographical area

A study published by Ssepuuya *et al*. (2016) explored the impact that geographical area can have on edible insects. Findings indicated that even though an edible grasshopper species (*Ruspolia nitidula*) was subjected to various geographical locations, it did not result in significant differences in protein content. More recent published literature however accentuated the potential in which external factors such as geographical area can have on the nutritional content of edible insects (Akhtar & Isman, 2018).

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2.7.2 Processing method

Edijala *et al*. (2009) mentioned the possibility in which heat treatment (depending on the specific treatment, temperature and time combination), can alter the protein content. Megido *et al*. (2018) mentioned a less promising drying method. Pan fried *Tenebrio molitor* samples resulted in the lowest protein content when compared to vacuum-cooked, boiled and oven-cooked. It is suspected that the pan-frying method can reduce the protein digestibility of the sample. This can be attributed to the oxidation of pan-fried insects due to the increased lipid content. The oxidation process further restricts the enzymatic proteolysis of protein, as a result of the interaction between proteins and lipids (Megido *et al*., 2018).

2.7.3 Quantification process of insect protein

Literature often emphasises the ample amount of protein present in insects. It is however essential that the quantification process provides an accurate reflection of the true protein content[1] as it will inevitably play a role in global nutritional outcomes (Finke, 2007; Jonas-Levi & Martinez, 2017).

2.7.3.1 Chitin content consideration

Cornelius *et al.* (1976) indicated that insects contain chitin as part of their exoskeleton, which is poorly digested by humans. A study done by Dreyer & Wehmeyer (1982) further accentuated the findings where a high proportion (20.1%) of the dried mopane worms was indigestible. Other studies followed, which further explored the impact of indigestible matter of edible insects on the quantification of crude protein content (Dufour, 1987; Bukkens, 1997). Research has since established that chitin is digestible by chitinolytic enzymes in the human body but does not contribute to the protein content of insects (Paoletti *et al*., 2007; Belluco *et al*., 2013). The degutting process of mopane worm during processing has been proven to increase the crude protein content by as much as 10% (Madibela *et al*., 2009). The removal of the mopane worm insides therefore affects the proportion of crude protein compared to chitin present (Moreki *et al*, 2012).

Research published by Finke (2007; 2013) indicated that crude protein content is a good reflection of the total protein content of insects, as long as the amino acid profile and protein recovering process[2] are done accurately. Yi (2015) however indicated that the presence of chitin can lead to inaccurate amino acid profiles due to the level of nitrogen present.

2.7.3.2 Kjeldahl & Dumas method

The Kjeldahl and Dumas methods are examples of indirect determination methods where the nitrogen content of a product is quantified (Finke, 2007; Müller, 2017; Mæhre *et al*., 2018). The total

[1] True protein content: Sum of total nitrogen sources contributing to protein content i.e. total amino acid content (Finke, 2007)

[2] Protein recovery: $\frac{\text{(total amino acids + taurine)}}{\text{nitrogen} \times 6.25}$ (Finke, 2013).

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amount of nitrogen is then multiplied by a standardised nitrogen-to-protein conversion factor (Kp) of 6.25 to achieve the crude protein content (Oonincx, 2015). The exoskeleton of insects however naturally contains nitrogen which does not contribute to the amount of protein present. Earlier protein values may therefore have been established as too high (Janssen *et al*., 2017; Jonas-Levi & Martinez, 2017).

The main difference between the Dumas and Kjeldahl methods is the specific nitrogen sources which are measured. The Dumas method measures the total particulate nitrogen (TPN), which includes inorganic and organic nitrogen, whereas the Kjeldahl method only measures ammonia and organic nitrogen (Sáez-Plaza *et al*., 2013; Müller, 2017). Various studies have indicated that the Dumas method often yields a slightly higher protein content than that of the Kjeldahl method (Thompson *et al*., 2002; Jung *et al*., 2003). This may be attributed to the Kjeldahl method being unable to recover the total organic nitrogen content (Mariotti *et al*., 2008). Currently, the Kjeldahl method is still acknowledged by the AOAC International as the international reference method for protein determination (Sáez-Plaza *et al*., 2013). Jonas-Levi & Martinez (2017) accentuated that the Kjeldahl method is generally the method of choice for the protein determination of insects.

2.7.3.3 Kjeldahl and Dumas modifications

Various Kjeldahl and Dumas modifications have entered the market from when the first standard method was introduced. According to Kirk & Sawyer (1991), the macro-Kjeldahl is similar to the standard Kjeldahl method described by the AOAC. The principles of the macro and micro-Kjeldahl are the same, whereas with the macro method, large apparatus are needed (Sáez-Plaza *et al*., 2013). A crucial difference however between the micro- and macro-Kjeldahl methods, is the amount of sample that is needed. Large quantities of sample, between 0.5 – 5 g, are needed for the macro-Kjeldahl, whereas the micro method require smaller sample sizes (less than 0.25 g). The amount and number of samples utilised during Kjeldahl's digestion step will inevitably influence the total nitrogen that is retrieved (Sáez-Plaza *et al*., 2013). Micro-Kjeldahl methods have proven to provide accurate results if the small-scale apparatus are knowledgeably handled and the procedure is executed with great precision. The sample size and intense precision necessary for micro-Kjeldahl however often expands the opportunity for errors in the quantification process. This deems the micro-Kjeldahl method unacceptable as a reference method (Sáez-Plaza *et al*., 2013).

The spectrophotometric and colorimetric methods are further examples of modified Kjeldahl methods. Kirk & Sawyer (1991) mentioned that due to the strict calibration needed, it is often difficult to establish a high accuracy level with the modified Kjeldahl. Previous studies indicated that contrasts in the results of modified Kjeldahl and standard Kjeldahl are often prevalent (Kirk & Sawyer, 1991). A paper published by William (1964) in which the colorimetric method was utilised, indicated that the colorimetric yielded similar results to that of the standard Kjeldahl method. A limitation however of

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the colorimetric method as highlighted by Amin & Flowers (2004), includes the possibility of altered results due to the interaction with other reagents and (in)organic compounds.

Lastly, the carbon, nitrogen and sulphur (CNS) analyser functions on the same principles of the Dumas combustion procedure (Thermo Scientific, 2010). A study published by Etheridge *et al*. (1998) indicated that the CNS analyser results compared favourably to the standard Kjeldahl method, only marginally exceeding that of the Kjeldahl method. This is in line with the main difference between the Dumas and Kjeldahl method previously mentioned (section 2.7.3.2).

2.7.3.4 Kp adjustment

The standard Kp of 6.25, was established as a robust estimate that food products on average contains 16% nitrogen (Owusu-Apenten, 2002; Mariotti *et al*., 2008). It is therefore clear that in using the same Kp for various protein determination methods with different protein recoveries, a wide range of results will be obtained (Müller, 2017).

Research done by Janssen *et al.* (2017) established individual Kp factors for three different species, *Tenebrio molitor* (mealworm beetle), *Hermetia illucens* (black soldier fly) and *Alphitobius diaperinus* (lesser mealworm). The results of the study emphasised the overestimation of protein content, as the Kp factors (4.67 – 4.85) for the insect species were lower than the standardised 6.25. A proposed solution is to decrease the standard Kp of 6.25 to 4.76 to compensate for the possible contribution of the chitin (non-protein nitrogen) to the protein content (Janssen *et al*., 2017).

The ideal situation would however be to determine the Kp of all insect species. Jonas-Levi & Martinez (2017) commented that with over 2 000 edible insect species consumed globally, it is not feasible to determine the Kp factors of each specie at each stage of development. An alternative quantification method is to subtract the proportion of non-digestible nitrogen (chitin content) from the total nitrogen (Hahn *et al*., 2018). According to Jonas-Levi & Martinez (2017), through the determination of N-glucosamine and carbohydrate hydrolysation process, the chitin content can effectively be measured. The crude protein content is then determined through multiplying the leftover nitrogen with the Kp factor (Jonas-Levi & Martinez, 2017).

2.7.3.5 Amino acid hydrolysis

Various studies have proposed that AA hydrolysis, a direct protein determination method, should be the protein determination method of choice (Owusu-Apenten, 2002; Mæhre *et al*., 2018). The AA hydrolysis as preferred protein determination method does however have limitations. There is a risk of a reduction in AA content during the determination process, as the hydrolysis step not only effectively hydrolysises peptide bonds but can affect the AA content (Mæhre *et al*., 2018). Secondly, the high financial costs associated with this quantification method is often a determining factor in why an alternative method is the preferred choice (Magomya *et al*., 2014).

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2.8 Market accessibility and demand for edible insects

It is estimated that by 2024, the global edible insect market will be worth more than US$ 722.9 million (Persistence Market Research, 2016). The annual trade of mopane worms alone, is estimated to be as high as US$ 59 million (Baiyegunhi *et al*., 2016). However, certain obstacles prevent harvesters or producers to fully benefit from the lucrative market. Accessibility to markets are often restricted due to lack of transport and long distances between harvesting and selling points (Dzerefos *et al*., 2014). The seasonal availability of insects can further act as a possible advantage or drawback as it will ultimately influence the supply and demand of products (Netshifhefhe *et al*., 2018). Currently, it is however still more profitable for small-scale producers to focus on livestock farming (Halloran *et al.*, 2016)

As insects become more of a delicacy, the price of these products can increase, especially due to the limited period of availability (Van Huis *et al*., 2013). This can lead to counterproductive results when the purpose is to encourage insect consumption. Currently insects are still more expensive than various other protein sources. In Mexico, ant pupae and larvae are sold for 200 US$/kg compared to 14 US$/kg for beef (Oonincx, 2015). During the process of urbanisation, the traditional lifestyles and consumption patterns are abandoned, and a more westernised lifestyle is adopted (Lim *et al*., 2009; Lehane, 2015). With the move to urban areas and the abandoning of traditional lifestyle, the harvesting of edible insects are often not sustainable. Dreams of increasing the quality of life during urbanisation is often unobtainable due to restrictions in entering the formal market, resulting in residents being forced to enter the informal market and reside in the less than ideal outer periphery areas of the city (Todaro & Smith, 2015). Without access to insect harvesting areas and restricted financial situation, this group are not able to purchase or gather insects. The target of reducing nutrient deficiencies can therefore be in vain, if a large group in dire need of nutrient rich food sources, such as insects, is left out of the equation.

Bessa *et al*. (2017) mentioned that sedulous and continuous collaboration is crucial in transforming the current marketing policies to achieve a greater inclusion of the target group. Increasing the amount of large-scale edible insect rearing facilities, can promote constant production, supply and improved quality through competitiveness in the market. This will inevitably play a role in insect products being more affordable and obtainable (Cortes Ortiz *et al*., 2016; Dobermann *et al*., 2017).

2.9 Consumer acceptance

Schlup & Brunner (2018) indicated nine factors that influence the acceptance of entomophagy. These factors include: “convenience orientation, the discernibility of insects in food, expected food healthiness, the need for familiarity, food neophobia, food technology neophobia, the perceived health benefits of meat, and the binary variables gender and prior consumption”.

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Baker *et al.* (2016) and Verbeke (2015) researched the role that food neophobia plays in insect consumption. According to these studies, food neophobia has a significant effect on the reluctance to taste and purchase edible insect products. More recently published studies however contradicts the statement in that food neophobia does not significantly affect the acceptance of edible insects (La Barbera *et al*., 2018; Schlup & Brunner, 2018). The lack of entomophagy acceptance is rather due to the "disgust" factor" associated with insects (Rumpold & Schlüter, 2013; Baker *et al*., 2016). Furthermore, the appropriateness of entomophagy often differs between various ethical and cultural groups (Deroy *et al*., 2015).

Consuming insects is often viewed as being inferior when compared to the beef consumption (Van Huis, 2003). However, if the Pedi tribe of South Africa have the choice between meat and insects, they would choose the latter (Oonincx, 2015). Insects are prepared in various manners, depending on personal taste. The citizens of the Vhembe district of Limpopo, often consume termites with maize meal porridge or prepared with other ingredients such as tomato and onions (Netshifhefhe *et al*., 2018).

A study done by Azzolini *et al*. (2018) indicated that consumers were less accepting of mealworm enriched wheat snacks when the amount increased from 10% to 20%. The lower percentage mealworm powder inclusion of mealworm yielded higher quality products than the higher inclusion. This can be contributed to the higher fat content of the mealworm, resulting in a decrease in dough expansion of the food products.

Informing consumers of insects' nutritional content and being less resource intensive compared to beef, will not automatically result in higher acceptance levels (Deroy *et al*., 2015). The standardisation of the quality and safety aspects of edible insect products are crucial to increase consumption rates (Behre *et al*., n.d., Halloran *et al*., 2016; Wilkinson *et al*., 2018). The involvement of supermarkets in marketing of edible insect products can promote uniform quality products, due to the stricter food safety and quality regulations in place (Halloran *et al*., 2016).

Tan *et al*. (2015) proposed a shift in the current focus of consumers not yet accustomed to insect consumption to population groups already practising entomophagy. Furthermore, the geographical area of consumer acceptance of edible insects, must be considered (Halloran *et al*., 2016). Citizens from developing countries, such as Kenya, are already accustomed to consuming insects. The processing of edible insects will therefore not significantly result in higher consumer acceptance (Alemu *et al*., 2017b). Incorporating insects into food products to reduce the visibility, will therefore most likely aid consumer acceptance in developed countries (Tan *et al*., 2015; Azzolini *et al*., 2018; Wilkinson *et al*., 2018).

Various trends influencing the process of entomophagy have been reported. Increasing the convenience factor, whether it involves online shopping or minimal preparation needed, can aid in the rise of edible insect popularity (Verbeke, 2015; Manuell, 2016). Furthermore, the noticeable increase in investment into the edible insect industry will further prompt entomophagy popularity

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(Tan *et al*., 2015; Manuell, 2016). Lacey (2016) included that continuous exposure and influence from social media and well-known public figures will play a role in increasing entomophagy.

2.10. Conclusion

Edible insects have been identified as a potential food source to assist in meeting the population's nutritional demands. Through the literature, it is evident that the nutritional content of edible insects frequently consumed in South Africa, is favourable in contributing to the RDA of nutrients often deficient in the population's diet. It can therefore be deemed beneficial to further analyse the insect's nutritional content.

The acceptance of insects will likely vary between specific geographical areas in South Africa. Edible insects as potential food source may be more readily accepted in certain areas where they already form part of population groups' diet and low in other areas where entomophagy is still unestablished. Decreasing the visibility of the insects through food product inclusion, is a proposed solution to increase acceptance of consumers not yet accustomed to entomophagy.

The current reality is however that the price of insect-containing food products remarkably exceeds that of animal-based protein sources. The process of commercialisation through mass-rearing facilities can potentially assist in reducing product prices. Through the literature it is however evident that to ensure the successful establishment of an insect rearing facility, various aspects need to be taken into consideration. Examples of these aspects include the type of insect, the feeding and specific environmental conditions necessary and the potential impact on the surrounding ecosystem if a non-native reared insect is released. In exploring potential edible insects for mass-rearing purposes, it would therefore be sensible to either select indigenous edible insect species or species already accustomed to the South African environment which will not threaten the existing ecosystem.

Research gaps regarding entomophagy however still exist. Concerns include possible allergic reactions, microbiological and heavy metal contamination. Increased research and implementation of food safety systems will be needed to produce safe food and improve consumer acceptance. Increased emphasis should be placed on the quantification methods of insect protein content. It is essential to produce reliable results to determine how insect protein can realistically contribute to meeting the population's nutritional needs**.**

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Chapter 3: Materials and Methods

The methods and literature which were identified during the research assignment were purposefully selected to achieve the outlined objectives. The primary objective included exploring the potential of edible insects in reducing the severity of the most prevalent nutrient deficiencies in South Africa.

Critical assessment and analysis of existing literature formed the primary method of data collection for this research assignment. The advantages of following a desktop research approach have been numerously highlighted in previous published works. The benefits of this approach include increased cost-effectiveness, convenience, access to published literature and exemption of ethical clearance (Tripathy, 2013; Cheng & Phillips, 2014).

As is the case with every research method, when acknowledging the advantages, it is essential to also include the disadvantages of the specific method. A limitation pertaining to the collection of existing literature for this research assignment, is the dependency on previously published literature as main data source (Johnston, 2014). Only the data presented in the existing literature could be utilised and the potential of incomplete or outdated data occurring, is a possibility (Cheng & Philips, 2014). In addition to the aforementioned, the possibility exists of certain usable literature sources being excluded from the study. Although the search process included a range of databases and a manual search, the probability of additional literature sources being overlooked cannot be completely eliminated.

The following methods describes the process and logical path that was followed during this research assignment. The identified equations stipulated throughout the methods, serves as an explanation and guide on the logical thought process employed in determining the results.

3.1 Nutrient deficiencies in South Africa

The first step in achieving the primary objective, comprised of determining the magnitude of nutrient deficiencies in South Africa. In viewing the nutrient deficiency statistics, essential insight was obtained on the severity of the situation and assists in placing the focus on the nutrients of highest concern.

The prevalence of nutrient deficiencies in South Africa was assessed through the obtainment of statistics and literature sources including Caulfield & Black (2004); the National Food Consumption Survey – Fortification Baseline (NFCS-FB): South Africa, 2005; the South African National Health and Nutrition Examination Survey (SANHANES-1) of 2013 (Shisana *et al*., 2013), Harika *et al*., 2017 and Charlton *et al*., 2018b.

3.2 Nutrient content of South African staple food products

In establishing the nutrient content of South African staple food products, valuable knowledge was obtained on which nutrients are sufficient and which are lacking in quantity. Investigation on the

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potential contribution of staple food consumption to the nutrient deficiency rate, aided in reaching the objective.

The Medical Research Council (MRC) FoodFinder3, a nutritional analysis computer programme, was identified as primary source of nutritional information for South African staple food products. Consideration must however be given to the fact that MRC FoodFinder3 (Medical Research Council, 2002), is unable to provide precise nutrient values of food products. The presented values therefore reflects the estimated average of these products (Wolmarans & Danster, 2008).

Through research, the most frequently consumed food products in South Africa were identified as maize meal and wheat bread (NFCS-FB, 2007; Ronquest *et al*., 2015). The most frequent procured types of maize meal in South Africa include sifted raw (white), followed by special (enriched), super raw (white) and lastly special raw (white) (NFCS-FB, 2007). The fortification legislation that forms part of the Foodstuffs, Cosmetics and Disinfectants Act (Act No. 54) of 1972, indicates mandatory fortification of the four most procured forms of maize meal. Following this information, the decision was made to include all maize meal forms as stipulated previously.

The initial MRC FoodFinder3 (Medical Research Council, 2002) search included all products containing the words “wheat”, “bread” and “maize”. The individual recipes of the respective products containing the nutrients per 100g raw product were then exported. During the search process, it however became prevalent that the MRC Foodfinder3 database (Medical Research Council, 2002) only contains the nutritional content of fortified white and brown bread or breadrolls. For the purpose of this research project, it would be more beneficial to assess the nutritional content of staple food products before fortification to identify potential paucity of specific nutrients. Considering this aspect, the decision was made to consult additional sources including the Foodstuffs, Cosmetics and Disinfectants Act, 1972 regarding the Regulations relating to the Fortification of Certain Foodstuffs (Foodstuffs, Cosmetics and Disinfectants Act, 2016).

As staple food products forms a pivotal part of the South African diet, it was deemed necessary to establish the significance of these products in meeting the RDA of micronutrients and daily requirements of amino acid (AA) (NFCS-FB, 2007). Thus, the micronutrient and amino acid (AA) content of the staple food products, were compared and expressed as a percentage of the daily requirement for adults. The micronutrient value of staple food products is expressed as %RDA of specific micronutrient according to equation 3.1. The RDA of the specific micronutrient (mg.100g^{-1} or µg.100g^{-1} product) in equation 3.1 was obtained from Table 3.1.

$$\frac{\text{Specific micronutrient value of staple food product (mg or µg. 100g}^{-1}\text{ product)}}{\text{RDA of specific micronutrient (mg or µg. 100g}^{-1}\text{ product)}} \times 100\% \qquad \textbf{(equation 3.1)}$$

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Table 3.1 The RDA of micronutrients, iron, zinc, iodine, folic acid and vit A, for adults (19 – 50 years).

	Micronutrients[a]								Reference
	Fe[b]		Zn[b]		I[c]	Vit B9[c]	Vit A[c]		
	M[d]	F[e]	M[d]	F[e]	M[d] & F[e]	M[d] & F[e]	M[d]	F[e]	Mahan & Raymond, 2017
RDA[f]	8	18	8	11	150	400	700	900	

[a]Abbreviations: **Fe**: Iron; **Zn**: Zinc; **I**: Iodine: **Vit B9**: Vitamin B9 / Folic Acid; **Vit A**: Vitamin A. [b]Given in mg/100g product; [c]Given in µg.100g^{-1} product**;** [d]M: Male; [e]F: Female; [f]RDA: RDA of adults between 19 – 50 years.

The AA content of staple food products was expressed as % contribution to daily AA requirement according to equation 3.2. The daily requirement of AA (mg.g^{-1} protein) in equation 3.2 was obtained from Table 3.2.

$$\frac{\text{AA value of staple food product (mg. g}^{-1}\text{ protein)}}{\text{Daily requirement of AA (mg. g}^{-1}\text{ protein)}} \times 100\% \qquad \textbf{(equation 3.2)}$$

Table 3.2 Amino acid (mg/g protein) requirements of adults older than 18 years of age.

	AA[a] (mg.g^{-1} protein)										Reference
	CYS	HIS	ILE	LEU	LYS	MET	PHE +TYR	THR	TRP	VAL	
Requirement[b]	6	15	30	59	45	16	38	23	6	39	WHO/FAO/UNU Joint Expert Consultation, 2007

[a]Abbreviations: **CYS**: Cysteine; **HIS**: Histidine; **ILE**: Isoleucine; **LEU**: Leucine; **LYS**: Lysine; **MET**: Methionine; **PHE**: Phenylalanine; **TYR**: Tyrosine; **THR**: Threonine; **TRP**: Tryptophan and **VAL**: Valine. [b]Requirement for adults (male and female) older than 18 years.

3.3 Identification of edible insects frequently consumed in South Africa

Research has indicated the potential of edible insects supplementing staple food products due to their favourable nutrient content (Bukkens, 1997). It was therefore decided to further investigate the nutrient content of edible insects. Only insects already consumed in South Africa were included due to familiarity factor (which can aid consumer acceptance) and the insects not acting as a potential threat to the existing ecosystem (Verbeke, 2015; Van Huis & Oonincx, 2017).

The primary source used to identify the insects consumed in South Africa, consisted of the list supplied by Van Huis (2003). The list of edible insects was then further compared with the extensive "Worldwide list of recorded edible insects" compiled by De Jongema (2017). Any additional insect species was added. Lastly, a database search, including Scopus, Science Direct, Medline, CAB Extracts, Web of Science and SA ePublications, was conducted. The following key words were used during the search: "edible insects"; "insect consumption", "entomophagy" and "South Africa".

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3.4 Literature search strategy

A systematic approach was followed during the literature search strategy. In adhering to the aforementioned technique, the occurrence of bias during the data collection process was minimised (Mallett *et al*., 2012). Fig 3.1 illustrates the layout and general overview of search strategy process and is roughly based on the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) flow diagram (Moher *et al*., 2009).

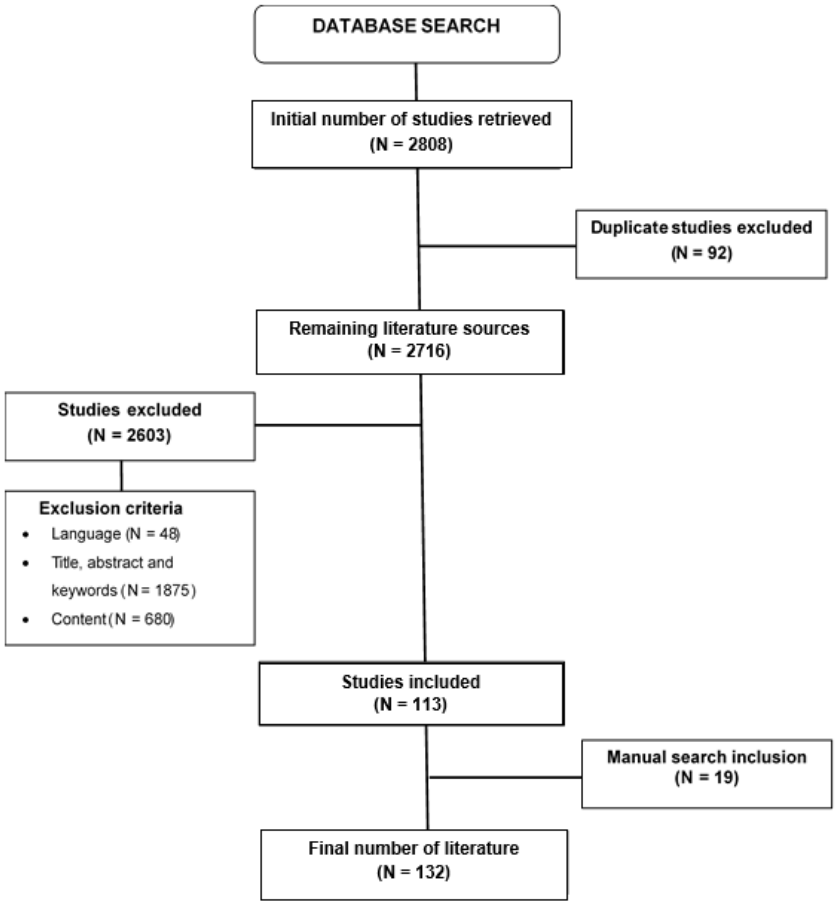

Figure 3.1 Logical flow illustrating the literature search strategy in acquiring relevant data on edible insect nutrient content. Adapted from the PRISMA flow diagram (Moher *et al*., 2009).

3.4.1 Data bases

The following databases were selected as primary search engines: CAB Extracts, Pubmed, SA ePublications, Science Direct, Scopus, Web of Science and JSTOR. The search process for each insect species through the aforementioned databases, occurred from 11 July to 17 July 2018. During the search duration, the relevant literature sources published before the specified time period, were identified.

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3.4.2 Inclusion and exclusion criteria

Table 3.3 indicates the inclusion and exclusion criteria used to select the scientific references for the compiling the edible insects' nutritional content. As with Fig 3.1, the structuring of Table 3.3 was retrieved from the PRISMA flow diagram (Moher *et al*., 2009).

3.4.2.1 Keywords

The specific keywords selected during the database are stated as follows: (*Species name*) AND (nutritional OR nutrition OR protein* OR energy* OR vitamin* OR mineral*) AND (entomophagy OR edible insect OR insect consumption).

Each species was individually searched for through all the databases. The total initial keywords search (KS) as indicated by Table 3.3 is thus the sum of the results (N = 2 808) from the databases. Each result was then analysed and included or excluded according to the specific criteria.

3.4.2.2 Duplicates

Any articles that were already identified and included during a previous database search were labelled as a duplication (D) and therefore excluded from further analyses. During the search, 92 articles were identified as duplicates.

3.4.2.2 Language

English was selected as the primary language (L) and any articles not complying with the language specifications, were excluded (N = 48).

3.4.2.3 Title, abstract and keywords

Each article's title, abstract and keywords (T,A,K) were assessed to establish whether any nutritional values (protein, iron, zinc, vitamin A, folate, iodine or amino acid profiles) of the specified species were measured (Table 3.3). A total of 1 875 articles did not meet the criteria and were exempted from further analysis.

3.4.2.3 Results and Discussion

The content including results and discussion (R, D) of each of the remaining articles were then further assessed. Only the articles that contained the specific nutritional values in question were included. Furthermore, during the content screening, any processed insects were excluded from further analysis. After the results and discussion screening, a further 680 articles were disqualified.

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Table 3.3 Systematic approach followed during the database search of the edible insects consumed in South Africa

Order	Family	Species	Common name	Life stage	KS[a]	Excluded				Included		FN[h]
						D[b]	L[c]	T, A, K[d]	R, D[e]	K[f]	HS[g]	
Coleoptera (Beetles)	**Curculioniae**	*Polyclaeis equestris*[1]	weevil	adult	0	0	0	0	0	0	0	0
		Polyclaeis plumbeus[1]	weevil	adult	0	0	0	0	0	0	0	0
	Buprestidae	*Sternocera orissa*[1]	jewel beetle	adult	8	0	0	3	3	2	0	2
		Thrincopyge alacris[2]	metallic wood-boring beetle		0	0	0	0	0	0	0	0
	Cerambycidae	*Mallodan downesi*[1]	long-horned beetle	larva	2	0	0	1	1	0	0	0
	Scarabaeidae	*Oryctes boas*[1]	rhinoceros beetle	larvae	17	2	0	6	6	3	0	3
		Oryctes monoceros[1]	rhinoceros beetle	larvae	18	0	1	4	10	3	0	3
		Oryctes owariensis[1]	rhinoceros beetle	larvae	2	0	0	0	1	1	0	1
	Tenebrionidae	*Tenebrio molitor*[3]	mealworm	larvae	468	27	14	252	158	17	3	20
Hemiptera (True bugs)	Tessaratomidae	*Encosternum delegorguei*[4]	edible stink bug	larvae / adult	53	10	0	14	24	5	0	5
Hymenoptera (Sawflies, ants, bees, wasps)	Formicidae	*Carebara vidua*[1]	African thief ant	winged adult	19	1	0	6	9	3	0	3
		Carebara lignata[4]			6	2	0	1	3	0	0	0
	Apidae	*Apis mellifera*[1]	honey bee	bee brood*	1138	2	2	1027	100	7	1	8

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Table 3.3 (Continued)

Order	Family	Species	Common name	Life stage	KS[a]	Excluded				Included		FN[h]
						D[b]	L[c]	T, A, K[d]	R, D[e]	K[f]	HS[g]	
		Trigona togoensis[2]			0	0	0	0	0	0	0	0
Blattodea (Cockroaches and termites)	Termitidae	*Odontotermes badius*[1]	fungus-growing termite	alate	11	0	0	7	3	1	0	1
		Odontotermes capensis[4]			8	0	0	3	5	0	0	0
		Macrotermes falciger[4]	roasted winged termites	alate	54	3	2	26	20	3	1	4
		Macrotermes natalensis[5]	fungus-growing termite	alate	33	3	1	13	10	6	0	6
		Macrotermes swaziae[1]	fungus-growing termite	alate	2	0	0	0	2	0	0	0
		Macrotermes michaelseni[5]	mound-building termite		10	0	0	6	4	0	0	0
		Trinervitermes trinervoides[1]	Harvester termite	adult	1	0	0	0	0	1	0	1
		Termes fatale[2]			52	0	0	48	4	0	0	0
	Hodotermitidae	*Microhodotermes viator*[1]	Southern Harvester termite	flyer nymphs	4	0	0	0	4	0	0	0
Lepidoptera (Caterpillars of moths and butterflies)	Lasciocampidae	*Bombycomorpha pallida Distant*[1]	pepper tree moth	larva	2	0	0	0	2	0	0	0
		Gonometa postica[1]	dark chopper	pupae	2	0	0	0	2	0	0	0
	Saturniidae	*Cirina forda*[1]	pallid emperor moth	larva	69	7	3	23	26	10	4	14

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Table 3.3 (Continued)

Order	Family	Species	Common name	Life stage	KS[a]	Excluded				Included		FN[h]
						D[b]	L[c]	T, A, K[d]	RD[e]	K[f]	HS[g]	
		Gonimbrasia belina[1]	mopane worm	larva	95	6	1	32	45	11	2	13
		Imbrasia ertli[6]	African moth larva	larva	7	0	1	0	2	4	0	4
		Imbrasia epimethea[6]	African moth larva	larva	16	3	2	4	4	3	0	3
		Gynanisa maja[1]	speckled emperor larva	larva	14	0	2	4	6	2	1	3
		Gynanisa ata[7]			1	0	1	0	0	0	0	0
		Argema mimosae[4]	African moon moth		6	0	0	4	2	0	0	0
		Bunaea alcinoë[4]	cabbage tree emperor moth	larva	19	2	2	2	11	2	1	3
		Melanocera menippe[4]	chestnut emperor	larva	1	0	0	0	1	0	0	0
		Heniocha apollonia[4]	southern marbled emperor		2	0	1	1	0	0	0	0
		Micragone cana[4]		larva	2	0	1	0	1	0	0	0
		Urota sinope[4]	tailed emperor	larva	2	0	1	0	1	0	0	0
	Sphigidae	*Agrius convolvuli*[1]	convolvulus hawk moth	larva	11	0	0	6	5	0	1	1
	Brahmaeidae	*Dactyloceras lucina*[4]	red spot moth		2	0	0	2	0	0	0	0

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Table 3.3 (Continued)

Order	Family	Species	Common name	Life stage	KS[a]	Excluded				Included		FN[h]
						D[b]	L[c]	T, A, K[d]	R, D[e]	K[f]	HS[g]	
	Eupterotidae	*Hemijana variegata*[4]		larva	10	3	0	2	3	2	0	2
Orthoptera (Crickets, locusts and grasshoppers)	Acrididae	*Locustana pardalina*[1]	brown locust	adult	12	0	0	6	6	0	0	0
		Locusta migratoria[2]	migratory locust	adult	220	4	5	160	48	3	2	5
		Nomadacris septemfasciata[1]	red locust	adult	17	0	1	8	7	1	1	2
		Schistocerca gregaria[4]	desert locust	adult	142	2	4	94	40	2	0	2
		Acanthacris ruficornis[6]	garden locust		16	0	2	11	3	0	0	0
	Tettigoniidae	*Ruspolia differens*[6]	katydid	adult	27	1	0	6	18	2	2	4
	Pyrogomorphidae	Zonocerus elegans[1]	coffee locust	nymph, adult	9	0	0	7	2	0	0	0
		Zonocerus variegatus[7]	variegated grasshopper	adult	47	2	0	22	17	6	0	6
		Phymateus viridipes[4]	green bush locust		1	0	0	0	1	0	0	0
Diptera (Flies)	Stratiomyidae	*Hermetia illucens*[3]	black soldier fly	larva	150	12	1	64	60	13	0	13
Total					**2808**	**92**	**48**	**1875**	**680**	**113**	**19**	**132**

[a] KS: Initial keyword search; [b] D: Duplicates; [c] L: Language; [d] T, A, K: Title, Abstract, Keywords; [e] R, D: Results, Discussion; [f] K: Keywords; [g] Hand search; [h] FN: Final number of articles included

References: [1]Van Huis, 2003; [2]Mitsuhashi, 2017; [3]Bessa, 2016; [4]Jongema, 2017; [5]Netshifhefhe *et al*., 2018; [6]Kelemu *et al*., 2015; [7]Bernard & Womeni, 2017.

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4.2.5 Manual Search

Finally, a manual hand search (HS) was conducted through Google Scholar of each species and consisted of the specified key words. Any results acquired from the manual hand search which passed the criteria, were included (N = 19).

3.5 Edible insects' nutrient content

The preceding steps led to this section which formed the vocal part in achieving the main objective. In obtaining the nutrient content of edible insects frequently consumed in South Africa, insects high in nutrients often devoid in staple food products were identified. The final number (FN) of insects which contained the nutrients in question, was established as 132 (Table 3.3). Only the remaining 132 articles were utilised in reporting the succeeding results.

The micronutrient values (vitamin A, zinc, iron, iodine and folate) obtained from the respective articles were tabulated for each species. In further investigating the edible insects' nutritional content and determining the contribution to the RDA, the outlined primary sub-objective of identifying edible insects high in nutrients often devoid in staple foods, can be achieved.

The minimum (Min) and maximum (Max) values, mean and standard deviation (Mean ± SD) of each micronutrient for the individual species was established. The specified micronutrient content of the individual insects were then expressed as the percentage RDA of adults (male and female) between 19 – 50 years. Micronutrient value of edible insects expressed as %RDA of specific micronutrient according to equation 3.3. The RDA of the specific micronutrient (mg or µg.100g^{-1} product) in equation 3.3 was obtained from Table 3.1.

$$\frac{\textbf{Specific micronutrient value of insects (mg or µg. 100g}^{-1}\textbf{ product)}}{\textbf{RDA of specific micronutrient (mg or µg. 100g}^{-1}\textbf{ product)}} \times \mathbf{100\%} \qquad \textbf{(equation 3.3)}$$

The same procedure was followed during the tabulation of the AA content of the edible insects. The AA content of staple food product expressed as percentage (%) contribution to daily AA requirement according to equation 3.4. The daily requirement of AA (mg.g-1 protein) in equation 3.4 was obtained from Table 3.2.

$$\frac{\textbf{[3]AA value of insects (mg. g}^{-1}\textbf{ protein)}}{\textbf{Daily requirement of AA (mg. g}^{-1}\textbf{ protein)}} \times 100\% \qquad \textbf{(equation 3.4)}$$

[3] AA values (mg. g^{-1} protein) are based on protein values in Table 3.4.

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Table 3.4 Mean protein values (%w/w) of specified edible insects

	Mean Protein Value (%w/w)	References
Sternocera orissa	[1]N/A	Shadung *et al*., 2012
Oryctes monoceros	36.45	Ifie & Emeruwa, 2011
Tenebrio molitor	47.89	Xingqian *et al*., 1998; Ravzanaadii *et al*., 2012; Bosch *et al*., 2014; Yi, 2015; Hopley, 2016; Zhao *et al*., 2016; Ghosh *et al*., 2017; Janssen *et al*., 2017
Encosternum delegorguei	35.45	Musundire *et al*., 2016a
Apis mellifera	34.55	Finke, 2005; Bednářová *et al*., 2013; Ghosh *et al*., 2017
Macrotermes falciger	42.53	Phelps *et al*., 1975; Siulapwa *et al*., 2014
Cirina forda	55.43	Igbabul *et al*., 2014; Inje *et al*., 2018
Gonimbrasia belina	54.76	Ohiopehai, 2006; Ekpo, 2011
Imbrasia ertli	48.66	Santos Oliveira *et al*., 1976
Imbrasia epimethea	70.80	Lautenschläger *et al*., 2017
Gynanisa maja	55.92	Siulapwa *et al*., 2014
Locusta migratoria	65.90	Purschke *et al*., 2018
Schistocerca gregaria	76.00	Zielińska *et al*., 2015
Ruspolia differens	45.823	Siulapwa *et al*., 2014; Fombong *et al*., 2017
Zonocerus variegatus	[2]N/A	Adeyeye, 2005
Hermetia illucens	33.58	Finke, 2005; Janssen *et al*., 2017; Liland *et al*., 2017; Liu *et al*., 2017

[1]N/A: Protein content not available. The amino acid analysis was utilised to establish the protein content after hydrolysis extraction, pre-column derivatisation, HPLC separation and fluorescence detection (Shadung *et al*., 2012).

[2]N/A: Crude protein was determined but not specified in publication (Adeyeye, 2005).

3.6 Protein quantification process

The protein content of food products is utilised in various incidences including nutrient content potential claims and determination of contribution to %RDA (Payne *et al*., 2016). Incorrect protein estimations can result in inadequate intake levels, especially when the reported value exceeds that of the true value (Jonas-Levi & Martinez, 2017). Accurate protein determination is therefore crucial to providing the actual protein content as stated.

The protein content of the edible insects was obtained through the initial database search. Due to a lack of studies conducted in South Africa in determining the nutritional content of frequently consumed insects, the decision was made to collect studies on the nutritional information of the

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same insect species even though differences in geographical areas may be present (Cheng & Phillips, 2014).

The various protein values obtained from the articles were tabulated and grouped according to the specific species that was measured. In each study, the corresponding protein quantification method was identified as indicated in the original literature source. Additional parameters (geographical area, life stage and preparation method) as stated in the original literature sources, were included.

3.7 Market accessibility and demand for edible insects

The viability of edible insects as complementary food source (sub-objective) was investigated. Even if edible insects are a favourable nutrient source, the contribution to reaching nutritional requirements will be negligible if "adequacy" and "accessibility" domains are not sufficient (De Schutter & Vanloqueren, 2011).

3.7.1 Edible insect market value compared to global processed meat market

In considering the aforementioned, the projected growth of the global processed meat and edible insect market, between 2018 – 2022, was investigated. Currently, the global processed meat market occupies a much greater proportion of the total food value chain than the edible insect market. The comparison of the global processed meat and edible insect market would therefore not have yielded significant results from which conclusions or recommendations could have been drawn. The decision was therefore made to establish the value of the edible insect market through determining the ratio between the edible insect and global processed meat market. Values were obtained from Zion Market Research (2017) and Statista (2018). For every US$1 billion the global processed meat market is worth, it was determined how much the edible insect market (US$ 100 000) would be worth within the same year. The ratio was determined using equation 3.5.

Global processed meat market (US$ 1 billion) : Global edible insect market (x)

$$\frac{\text{US \$ 1 billion}}{\text{US \$ 1 billion}} : \frac{x}{\text{US\$ 1 billion}} \qquad \textbf{(equation 3.5)}$$

3.7.2 Protein-price comparisons of animal-based sources and termites

The affordability factor will play a crucial role in determining the accessibility of edible insects. The price of food products often plays a crucial role in why a certain food product is chosen above another, especially in low-income groups (French *et al*., 2010). For this reason, comparisons between various protein sources, chicken breasts fillets, chicken livers, French polony, beef mince and termites (Netshifhefhe *et al*., 2018), were made in terms of the edible protein content (%) and the price in South African Rand per kg (ZAR/kg) product.

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The animal-based sources prices reflect the average retail price of three major South African supermarket outlets as on 17 September 2018. Free-range chicken breast fillets were excluded from the protein-price comparisons, due to the inconsistent availability in supermarket outlets on the identified date. MRC Foodfinder 3 (Medical Research Council, 2002) was utilised to obtain the protein content of food products. To establish the cost (ZAR/kg) to acquire 1% protein for each protein food source, equation 3.6 was used.

$$\frac{\text{ZAR/kg}}{\text{\%protein}} \qquad \textbf{(equation 3.6)}$$

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Chapter 4: Results and Discussion

In 1996, during the World Food Summit in Italy, it was concluded that food security is achieved when "all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life" (Erickson, 2008). To achieve future food security and adhere to all the dimensions, larger emphasis should be placed on the critical evaluation of the current food production processes. Statistics indicate that even though food production increased by 12% in sub-Saharan Africa in the past 20 years, more than 220 million people are still undernourished (May, 2016).

During times of economic hardships, citizens of especially lower income groups, will resort to less expensive food products, such as staple foods (Umberger, 2015). This is evident in South Africa where maize is the largest food commodity consumed on an annual basis (Ronquest-Ross *et al*., 2015). Research has however indicated that maize meal and bread are not ideal sources of micronutrients, often insufficient in essential nutrients such as iron, zinc and vitamin A (Oriz-Monasterio *et al*., 2007). Furthermore, maize meal and bread are known to contain limited amounts of lysine and tryptophan (Bukkens, 1997). Increasing the consumption of staple food products therefore poses as a potential contributing factor to the micronutrient deficiency intake. It is thus essential that in reducing the micronutrient deficiencies in South Africa, citizens need accessible and consistent food sources sufficient in providing the lacking micronutrients.

The nutritional profile, sustainable development and high production rate of edible insects is continuously being recognised and deems edible insects to be a viable contender in the search for alternative food sources (Oonincx *et al*., 2010). Especially the nutritional content of edible insects is increasingly being investigated and results have indicated favourable vitamin, mineral and amino acid contents (Xiaoming *et al*., 2010; Van Huis *et al*., 2013; Kouřimská & Adámková, 2016). Drawing from the aforementioned information, this chapter further investigated the potential use of edible insects as a supplementary or complimentary food source to reduce the most prevalent nutrient deficiencies in South Africa, while in adhering to the food security dimensions, namely "utilisation", "availability", "adequacy" and "accessibility" in a sustainable manner (Barrett, 2010).

In fulfilling the outlined objectives, the most significant nutrient deficiencies in South Africa, the nutritional content of staple food products and the potential of contributing to the rate of micronutrient deficiencies was explored. Parallels were then drawn between the most prevalent micronutrient deficiencies and insects high in those specific nutrients. Through this, valuable insight can be gathered on edible insects acting as food sources in meeting the population's nutritional needs.

Furthermore, the protein determination method for edible insects has been scrutinised for yielding inaccurate results. In acknowledging the pivotal factor in producing reliable nutritional

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results, the various protein determination methods and corresponding protein results were compared.

Lastly, the market viability and accessibility of edible insects was explored. For insects to be considered as a viable contender in the food market, various factors such as consumer acceptance, sustainable production and correct pricing needs to be achieved.

4.1 Prevalence of nutrient deficiencies in South Africans

When viewing Table 4.1, it is noticeable that zinc is the most common deficiency, with between 49.30 – 73.90% of the population being affected. Mild iodine deficiency was recognised as the second most prevalent deficiency compared to the other deficiencies listed. Folate deficiency rates of adult females (16 – 35 years of age) were deemed the lowest deficiency rate between the deficiencies listed in Table 4.1.

The high zinc deficiency statistics is however a reflection of the AFR-E subregion grouping which comprises of a multitude of different countries[4] (Caulfield & Black, 2004). It is therefore impossible to precisely determine as to where South Africa falls within the range. It is evident, that even at the lowest value (49.30%), zinc deficiency is still considered the most common deficiency when compared to the other deficiencies in Table 4.1. This is however in contrast with global statistics, which identify iron deficiency as the most widespread deficiency (Bailey *et al*.,2015). A possible explanation to South Africa having a higher zinc deficiency rate than compared to the global statistics, is the high HIV/AIDS status which negativity affects absorption rate (Sneij *et al*., 2016; StatsSA, 2017).

A mild iodine deficiency, 34.90% for men and 41% for women, is the second most common deficiency in South Africa according to Table 4.1, followed by vitamin A deficiency (22%). The potential in querying the aforementioned statistics does exist, especially when considering previous criticism regarding iodine results. Due to the daily variation in the iodine content of urinary samples, the reliability of comparing iodine levels of urinary samples against standard reference values, have been questioned (Charlton *et al*., 2018b). The age range in which the statistics were published must also be considered. Iodine has the widest age range (18 – 90 years) of all the statistics, which could therefore result in a higher deficiency rate. As for the vitamin A deficiency rate, a lack in statistics for men corresponds with previous literature which acknowledges that statistics mainly indicate the vitamin A status of women and children (Bailey *et al*., 2015).

[4] The AFR-E subregion grouping includes the following countries: Botswana, Burundi, Central African Republic, Congo, Côte d'Ivoire, Democratic Republic of the Congo, Eritrea, Ethiopia, Kenya, Lesotho, Malawi, Mozambique, Namibia, Rwanda, South Africa, Swaziland, Uganda, United Republic of Tanzania, Zambia and Zimbabwe.

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Table 4.1 Prevalence of the deficiency rates (%) of nutrients often lacking in the South African adult population's (female and male) diet.

	Severity or type	Female (%)	Male (%)	Average (%)	Age group	Reference
Iron	-	16.00	N/A[a]	-	≥15 – 49 years	Harika *et al*., 2017
IDA[b]	-	10.00	N/A[a]	-	≥15 - 49 years	Harika *et al*., 2017
Anaemia	Mild	12.40	10.60	11.50	≥ 15 years	Shisana *et al*., 2013
	Moderate	8.50	1.50	5.00	≥ 15 years	Shisana *et al*., 2013
	Severe	1.10	0.20	0.65	≥ 15 years	Shisana *et al*., 2013
Zinc[c]	-	49.30 – 73.90		61.60	15 – 44 years	Caulfield & Black, 2004
Folate	-	0.10[d] / 0.20[e]	N/A[a]	-	16 – 35 years	NFCS-FB, 2007
Vitamin A	-	22.00	N/A[a]	-	≥15 - 49 years	Harika *et al*., 2017
Iodine	Mild	41.00	34.90	37.95	18 - 90 years	Charlton *et al*., 2018b
	Moderate	16.10	12.80	14.45	18 - 90 years	Charlton *et al*., 2018b

[a]N/A: Statistics not available

[b]IDA: Iron-deficiency anaemia

[c]Due to lack of statistics available for South Africa, statistics for AFR-E (Botswana, Burundi, Central African Republic, Congo, Côte d'Ivoire, Democratic Republic of the Congo, Eritrea, Ethiopia, Kenya, Lesotho, Malawi, Mozambique, Namibia, Rwanda, South Africa, Swaziland, Uganda, United Republic of Tanzania Zambia and Zimbabwe) subregion grouping were utilised.

[d]Serum folate deficiency

[e]Red blood cell folate deficiency

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According to the NFCS-FB (2007), the typical South African diet does not contain sufficient amounts of folate. When assessing Table 4.1, it became evident that folate was however the least prevalent deficiency amongst women. Mandatory fortification of staple food products has been postulated as one of the possible reasons for the decrease in folate deficiency rate in South Africa (Metz, 2013). However, when considering that fortification of staple food products in South African mills does not automatically result in the mandatory micronutrient quantities being met (Yusufali *et al*., 2012), other possible contributing factors must be explored. The provision of folate supplementation before and during pregnancy on national level can potentially aid in reducing or preventing folate deficiency (Reynolds, 2002; Rees, 2016) Furthermore, from Table 4.1 it is evident that the statistic regarding the folate status of South African males was not available. This corresponds with previously published literature highlighting the lack of folate deficiency statistics on a global level (McLean *et al*., 2008).

From the aforementioned, two main concluding marks were formed. Firstly, even though the severity of micronutrient deficiencies is acknowledged as a focal problem in South Africa, statistics and data (especially of men) are insufficient in illustrating the full extent of the situation. Secondly, food fortification has been eulogised for its in role in the diminishing of nutrient deficiencies. However, it is evident from Table 4.1 that nutrient deficiencies are still a great area of concern. The zinc deficiency rate is regarded as the most severe deficiency in South Africa compared to the other deficiencies listed in Table 4.1, followed by mild iodine deficiency and then vitamin A deficiency rate in females. According to the statistics from various sources, severe anaemia and folate deficiency have the lowest deficiency rates in South Africa. In acknowledging these findings, the necessity in exploring alternative routes in alleviating micronutrient deficiencies becomes evident.

4.2 Micronutrient content: Staple food products and edible insects

When drawing comparisons between the micronutrients from staple food products (Table 4.2) and edible insects (Table 4.3), distinct differences between the two tables are visible. The overall average micronutrient values of South African staple food products ranges between 8 $\mu g.100g^{-1}$ product for folic acid and 2.5 $mg.100g^{-1}$ product for iron. When viewing the overall micronutrient values in Table 4.3, the lowest mean value of edible insects is the vitamin A content of *Macrotermes natalensis* (2.56 $\mu g.100g^{-1}$) whereas *Ruspolia differens* have the highest value of 117.24 $mg.100g^{-1}$ product for iron. The wide range of micronutrient content between species evident in Table 4.3 is in line with findings of previously published research (Bukkens, 1997; Akhtar & Isman, 2018). Furthermore, it is noticeable that the more nutrient results which are included from various secondary sources, the larger the likelihood is of a higher standard deviation (often exceeding the mean values). This corresponds with Payne *et al*. (2016), who accentuated the impact which a variation in external factors, including diet composition, stage of development and preparation method, can have on the standard deviation of micronutrient values.

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Table 4.2 Micronutrient values of South African staple food products (wheat flour, bread and maize meal) expressed as a percentage contribution to .the RDA of adults (male and female).

	Iron (mg.100 g^{-1} product)			Zinc (mg.100 g^{-1} product)			Iodine (µg.100 g^{-1} product)		Folic acid (µg.100 g^{-1} product)		Vitamin A (µg.100 g^{-1} product)			Reference
	Value*	%RDA[a]		Value*	%RDA[a]		Value*	%RDA[a]	Value*	%RDA[a]	Value*	%RDA[a]		
		M[b]	F[c]		M[b]	F[c]						M[b]	F[c]	
White bread flour	1.35	16.88	7.50	0.80	10.00	7.27	-	-	8.00	2.00	0	0	0	Foodstuffs, Cosmetics and Disinfectants Act, 2016
Brown bread flour	2.50	31.25	13.89	1.90	23.75	17.27	-	-	51.00	12.75	0	0	0	Medical Research Council, 2002
White bread	1.25	15.63	6.94	0.7	8.75	6.36	-	-	15.00	3.75	0	0	0	Foodstuffs, Cosmetics and Disinfectants Act, 2016
Brown bread	1.80	22.50	10.00	1.35	16.88	12.27	-	-	23.00	5.75	0	0	0	Foodstuffs, Cosmetics and Disinfectants Act, 2016
Super MM[d]	0.65	8.13	3.61	0.58	7.25	5.27	-	-	8.00	2.00	0	0	0	Medical Research Council, 2002
Special MM[d]	1.20	15.00	6.67	1.53	19.13	13.91	-	-	42.00	10.50	23.00	3.29	2.56	Medical Research Council, 2002
Sifted MM[d]	1.50	18.75	8.33	1.67	20.88	15.18	-	-	29.00	7.25	12.00	1.71	1.33	Medical Research Council, 2002
Unsifted MM[d]	2.00	25.00	11.11	1.90	23.75	17.27	-	-	21.00	5.25	0	0	0	Medical Research Council, 2002

[a] %RDA: Nutrient value expressed as a percentage of the RDA as stipulated in Table 3.1; M: Male; [c] F: Female; [d] MM: Maize meal; *****Unfortified nutrient values.

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Table 4.3 Micronutrient values of edible insects consumed in South Africa expressed as a percentage contribution to the RDA of adults (male and female).

	Iron (mg.100 g^{-1} product)			Zinc (mg.100 g^{-1} product)			Iodine (µg.100 g^{-1} product)		Folic acid (µg.100 g^{-1} product)		Vitamin A (µg.100 g^{-1} product)		
	Mean ± SD[a]	%RDA[b]		Mean ± SD[a]	%RDA[b]		Mean ± SD[a]	%RDA[b]	Mean ± SD[a]	%RDA[b]	Mean ± SD[a]	%RDA[b]	
		M[c]	F[d]		M[c]	F[d]						M[c]	F[d]
Sterconera orissa[1]	84.64	1058.00	470.22	95.33	1191.63	866.64	-	-	-	-	-	-	-
Oryctes boas[2,3,4]	4.65 ± 4.05	58.13	25.83	0.41	5.14	3.73	-	-	-	-	8.58	1.23	0.95
Oryctes monoceros[2,5]	85.00	106.25	472.22	7.00	87.50	63.64	-	-	-	-	-	-	-
Oryctes owariensis[6]	20.26	253.25	112.56	7.89	98.63	71.73	-	-	-	-	-	-	-
Tenebrio molitor [7-15]	8.52 ± 9.38	106.50	47.33	10.37 ± 2.24	129.63	94.27	-	-	157.00	39.25	48.60	6.94	5.40
Encosternum delegorguei L[g; 16]	28.33	354.13	157.39	28.00	350.00	254.55	-	-	-	-	-	-	-
Encosternum delegorguei A[h; 17,18]	45.1 ± 35.21	563.75	250.56	33 ± 18.38	412.50	300.00	-	-	-	-	230.00	32.86	25.56
Carebara vidua[19]	10.69	133.63	59.39	5.69	71.13	51.73	-	-	451.00	112.75	767.00	109.57	85.22
Apis mellifera[2,3,20]	9.43 ± 13.66	117.88	52.39	1.60	20.00	14.55	-	-	-	-	12.44	1.78	1.38
Odontotermes badius[21]	9.60	120.00	53.33	8.23	102.88	74.82	-	-	-	-	172.00	24.57	19.11
Macrotermes falciger[22]	18.60	232.50	103.33	5.30	66.25	48.18	-	-	-	-	-	-	-
Macrotermes natalensis[2,3]	29.00	362.50	161.11	-	-	-	-	-	-	-	2.56	0.37	0.28

[g] L: Larvae

[h] A: Adult

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Table 4.3 (Continued)

	Iron (mg.100g^{-1} product)			Zinc (mg.100 g^{-1} product)			Iodine (µg.100 g^{-1} product)		Folic acid (µg.100 g^{-1} product)		Vitamin A (µg.100 g^{-1} product)		
	Mean ± SD[c]	%RDA[b]		Mean ± SD[c]	%RDA[b]		Mean ± SD[c]	%RDA[b]	Mean ± SD[c]	%RDA[b]	Mean ± SD[c]	%RDA[b]	
		M[c]	F[d]		M[c]	F[d]						M[c]	F[d]
Cirina forda[2, 7, 16, 23 - 31]	27.58± 26.86	344.75	153.22	74.17 ± 126.31	927.13	674.27	-	-	-	-	2.99	0.43	0.33
Gonimbrasia belina[13, 16, 28, 32, 33]	51.06 ± 35.45	638.25	283.67	17.95 ± 4.75	224.38	163.18	-	-	-	-	29.23 ± 11.42	4.18	3.25
Imbrasia ertli [13, 34, 35]	2.73 ± 1.27	34.13	15.17	-	-	-	-	-	6.80	1.70	47.3	6.76	5.26
Imbrasia epimethea[13, 36]	13.00	162.50	72.22	11.10	138.75	100.91	-	-	34.90 ± 39.74	8.73	45.65 ± 2.33	6.52	5.07
Gynanisa maja[16, 37]	20.13 ± 9.24	251.63	111.83	19.30	241.25	175.45	-	-	-	-	-	-	-
Bunaea alcinoë[38]	38.67	483.38	214.83	24.73	309.13	224.82	-	-	-	-	-	-	-
Agrius convolvuli[39]	18.56	232.00	103.11	6.74	84.25	61.27	-	-	-	-	-	-	-
Hemijana variegata[40]	-	-	-	-	-	-	-	-	-	-	20.00	2.86	2.22
Locusta migratoria[13, 40, 41]	9.64 ± 5.80	120.50	53.56	10.65 ±3.57	133.13	96.82	-	-	-	-	14.70	2.10	1.63

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Table 4.3 (Continued)

	Iron (mg.100 g^{-1} product)			Zinc (mg.100 g^{-1} product)			Iodine (µg.100 g^{-1} product)		Folic acid (µg.100 g^{-1} product)		Vitamin A (µg.100 g^{-1} product)		
	Mean ± SD[a]	%RDA[b]		Mean ± SD[a]	%RDA[b]		Mean ± SD[a]	%RDA[b]	Mean ± SD[c]	%RDA[b]	Mean ± SD[a]	%RDA[b]	
		M[c]	F[df]		M[c]	F[d]						M[c]	F[d]
Schistocerca gregaria[15]	8.38	104.75	46.56	18.60	232.50	169.09	-	-	-	-	-	-	-
Ruspolia differens[43 - 45]	117.24 ± 144.87	1465.50	651.33	14.63 ± 0.31	182.88	133.00	-	-	620.00 ± 395.98	155.00	1.57 ± 1.24	0.22	0.17
Zonocerus variegatus [2,3,26, 46 - 48]	8.00 ± 15.48	100.00	44.44	0.42 ± 0.42	5.25	3.82	-	-	-	-	6.82	0.97	0.76
Hermetia illucens[13,49 - 54]	75.01 ± 69.30	937.63	416.72	25.72 ± 25.76	321.50	233.82	18.42 ± 26.00	12.28	270.00	67.50	-	-	-

[a] Mean ± Standard deviation; [b] Mean expressed as percentage RDA as stipulated in Table 3.1; [c] M: Male; [d] F: Female

References: [1]Shadung *et al*., 2012; [2]Alamu *et al*., 2012; [3]Banjo *et al*., 2006; [4]Fasunwon *et al*., 2011; [5]Ifie & Emeruwa, 2011; [6]Assielou *et al*., 2015; [7]Barker *et al*., 1998; [8]Dobermann *et al*., 2017; [9]Ghosh et al., 2017; [10]Kim *et al*., 2017; [11]Kuntandi, 2018; [12]Ravzanaadii *et al*., 2012; [13]Williams *et al*., 2016; [14]Xingqian *et al*., 1998; [15]Zielińska *et al*., 2015; [16]Payne *et al*., 2015; [17]Musundire *et al*., 2016a; [18]Teffo *et al*., 2007; [19]Ayieko *et al*., 2016; [20]Finke, 2005; [21]Kenji *et al*., 2010; [22]Chulu, 2015; [23]Akinnawo & Ketiku, 2000; [24]Igbabul *et al*., 2014; [25]Khan, 2018; [26]Ogunleye, 2006; [27]Omotoso, 2006; [28]Onigbinde & Adamolekun, 1998; [29]Osasona & Olaofe, 2010; [30]Paiko *et al*., 2014; [31]Rumpold & Schlüter, 2013; [32]Dreyer & Wehmeyer, 1982; [33]Glew *et al*., 1999; [34]Mumba & Jose, 2006; [35]Santos Oliveira *et al*., 1976; [36]Kodonki *et al*., 1987; [37]Siulapwa *et al*., 2014; [38]Dauda *et al*., 2014; [39]Tiroesele *et al*., 2013; [40]Egan, 2013; [41]Mohamed, 2015; [42]Oonincx & van der Poel, 2011; [43]Fombong *et al*., 2017; [44]Kinyuru *et al*., 2009; [45]Kinyuru *et al*. 2010; [46]Ladeji *et al*., 2003; [47]Olaofe *et al*., 1998; [48]Sani *et al*., 2014; [49]Finke, 2013; [50]Liland *et al*., 2017; [51]Liu *et al.,* 2017; [52]Makkar *et al*., 2014; [53]Nyakeri *et al*., 2017; [54]Spranghers *et al*., 2016.

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During further exploration of the nutritional information of staple food products and that of edible insects, a similar trend in iodine content was identified. No iodine values for the staple food products was available, whereas with edible insects, only *Hermetia illucens*' iodine content was established in two respective studies (Spranghers *et al*., 2016; Liland *et al*., 2017), contributing 12.28% to the RDA of adults per 100 g product consumed. Findings by Oonincx (2015) also mentioned the frequent exclusion of iodine content determination during the nutritional analyses of edible insects. In acknowledging the various discrepancies regarding the iodine deficiency rate (Table 4.1), paucity in iodine values of staple food products (Table 4.2) and edible insects (Table 4.3), it is clear that a lack of information regarding the overall iodine content of food products and the iodine status of individuals exist (Harris, 2003; Foodstuffs, Cosmetics and Disinfectants Act, 2016; Charlton *et al*., 2018). Due to the limited amount of iodine values available in Table 4.2 and 4.3, it is impossible to draw any definite conclusions as to whether staple food products contain low amounts of iodine. As a result of the previously mentioned, it would therefore not be feasible to make any recommendations regarding the supplementation or complementation of these staple food products with iodine rich edible insects.

As is the case with the iodine content of staple food products, the vitamin A values of the majority of staple food products in Table 4.2 are not available, expect for special (23.00 µg.100g^{-1} product) and sifted maize meal (12.00 µg.100g^{-1} product). This is in agreement with multiple other studies categorising grain products as a less than ideal source of vitamin A (Duvenage & Schönfeldt, 2007; Dewettinck *et al*., 2008; Van Jaarsveld *et al*. 2015). *Carebara vidua* (African thief ant) can however contribute to adults' intake of vitamin A in contributing 85.22% of the female and 109.57% of the male's RDA per 100 g consumed (Ayieko *et al*., 2016). The second highest vitamin A values in Table 4.3 is those of the larvae of *Encosternum delegorguei*, containing 230 µg per 100g product (Teffo *et al*., 2007). The contribution to an adult's RDA of vitamin A for *E. delegorguei* (32.85% for males and 25.56% for females) when consumed per 100g, is however significantly lower than that of *C. vidua*. The remaining insects' vitamin A is even lower, inevitably resulting in the need to consume a higher amount to meet the RDA. The findings presented in Table 4.3 is consistent with literature which indicates the low overall vitamin A content of edible insects (Kouřimská & Adámková; 2016; Sánchez-Muros *et al*., 2014). In acknowledging the information available, it seems as if *E. delegorguei*, compared to the other edible insects in Table 4.3, is the only viable option to potentially contribute to the vitamin A content of staple food products. Further consideration and research is therefore needed to assess the suitability of edible insects in meeting RDA of vitamin A and potentially prevent or decrease deficiencies.

From Table 4.2, it is evident that unfortified staple food products cannot be viewed as significant sources of zinc, ranging between 0.58 mg.100g^{-1} product for super maize meal and 1.90 mg.100g^{-1} product for both unsifted maize meal and brown bread flour. Reasons postulated for the low zinc content of grain products, include the extensive milling process and presence of phytic acid in maize, which both result in a decreased zinc availability (Nuss & Tanumihardjo, 2010; Suri &

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Tanumihardjo, 2016). A multitude of edible insects however can potentially act as complimentary or supplementary food product or ingredient when considering the contribution to meeting the RDA of adults' zinc content. Consuming 100 g of *Sternocera orissa* results in meeting males' RDA of zinc content more than ten times and more than eight times for females (Shadung *et al*., 2012). Furthermore, Shadung *et al.,* 2012 highlighted the effect that the specific drying method can have on the zinc content of edible insects. It is therefore essential to consider the drying method of choice when including *S. orissa* as source of zinc to retain the maximum amount.

Considering the important role of iron in overall wellbeing and performance and the debilitating effect iron deficiencies have on population groups, it was deemed essential to further investigate the nutritional content of grain products and edible insects in South Africa (Shisana *et al*., 2013; Phatlhane *et al*., 2016). Insects have proven to be an excellent source of iron, which is often lacking in the human diet. The majority of the edible insect species in Table 4.3, have an iron content which can contribute 50% of the total RDA of adults when consumed per 100g product, with the exception of *Oryctes boas* (rhinoceros beetle)**,** 25.83% of women' RDA, and *Imbrasia ertli* (African moth larva), 34.13% of men's RDA and 15.17% of women (Santos Oliveira et al., 1976; Banjo *et al*., 2006; Mumba & Jose, 2006; Fasunwon *et al*., 2011; Alamu *et al*., 2012; Williams *et al*., 2016).

When compared to the iron content of staple food products, the edible insect contents are significantly higher. Brown bread flour has the highest iron content of 2.50 mg per 100g product compared to the other staple food products listed in Table 4.2. On the other hand, *Ruspolia differens* has an iron content of 117.24 mg per 100 g product (Kinyuru *et al*., 2009; Fombong *et al*., 2017) *Gonimbrasia belina* is further an example of an edible insect which contribute to more than the daily RDA of iron for adults (283.67% for females and 638.25% for males) when consumed per 100 g product (Dreyer & Wehmeyer, 1982; Onigbinde & Adamolekun, 1998; Siulapwa *et al*., 2014; Payne *et al*., 2015). This is in line with previously published research (Bukkens, 1997) regarding the favourable iron content of *Gonimbrasia belina*. However, unresolved concerns regarding the bioavailability of the iron content of edible insects exists (Doberman *et al*., 2017).

The folic acid content of insects was limited to a few studies. Drawing parallels between the nutritional content of staple food products and edible insects is therefore limited. Results of *Ruspolia differens,* part of the Orthoptera order, is in accordance with literature published by Rumpold & Schlüter (2013) accentuating the often-favourable folic acid content of the Orthoptera order. *Ruspolia differens* provides 150% of the RDA for folic acid (Kinyuru et al., 2009; 2010). A study done by Ayieko *et al*., 2016 (Table 4.2) indicated that *Carebara vidua* contains a significant 451.00 µg.100 g^{-1} product, resulting in more than 100% of the RDA of folic acid per 100 g product consumed. Although the availability of research regarding folic acid content of edible insects is limited, when comparing to the folic acid content of unfortified staple food products, the potential in assisting folic acid content exists. In stark comparison, the staple food products, when consumed per 100g, contribute to between 2.00 and 12.75% of the RDA of adults.

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In acknowledging the aforementioned information, it is evident that a multitude of edible insects in South Africa contain favourable nutritional contents. Various edible insects consumed in South Africa have the potential to contribute more than half or even exceed the RDA of micronutrients when consumed per 100 g product (Table 4.3). Smaller quantities of these insects can therefore be consumed and still reach half the adult RDA of micronutrients. *Sternocera orissa*, *Gonimbrasia belina*, *Ruspolia differens* and *Carebara vidua* are examples of such edible insects. To obtain 50% of the RDA of iron and zinc, men only need to consume 4.73 g of *S. orissa*, whereas only 10.63 g are necessary for women to meet half of their RDA of iron. *Gonimbrasia belina* contributes to 50% of the RDA of folic acid when 32.26 g of product is consumed. For the same amount, males would receive more than half and females 42% of their RDA of zinc. Furthermore*, Ruspolia differens* and *Carebara vidua* can also be viewed as significant sources of folic acid, as only 32.35 g of *R. differens* or 58.67 g of *C. vidua* needs to be consumed respectively to meet half of the RDA of folic acid for adults. Lastly, the same amount of *R. differens* (32.25 g), will supply more than four times the RDA of iron for males and more than twice the amount for females.

Taking into consideration the micronutrient content of the aforementioned edible insects and the quantities required to provide at least half of the RDA of nutrients, these edible insects can be identified as potentially viable contenders in acting as complimentary or supplementary food sources alongside grain products in South Africa.

4.3 Amino acid content comparison: Staple food products and edible insects

Numerous published works have accentuated the limiting AA content in staple foods (Bukkens, 1997; Dewettinck *et al*., 2008). Staple food products on average contributes between 3.56% (lysine) - 57.17% (cysteine) to the daily AA requirement per 100g product consumed (Table 4.4). Research has however indicated that due to the favourable AA content of edible insects, they could supplement the limiting AA's in staple food products (Kouřimska & Adámková, 2016). The edible insects' AA content encompasses a wider range than that of the staple food products, contributing between 22.05% (*Stenocera orissa*'s phenylalanine and tyrosine content) – 415.73% (*Ruspolia differens*' histidine content) of the daily AA requirement consumed per 100g product (Table 4.5). As with the micronutrient values in Table 4.3, it is evident that in certain incidences the standard deviation exceeds that of the average AA content (Table 4.5). Similar to the micronutrient values, as the amount of literature sources containing amino acid values of edible insects increases, so does the probability of obtaining a wider range of amino acid values. The same explanation provided for the high standard deviation compared to the mean micronutrient values is relevant to the standard deviation of edible insects' AA content. This can be contributed to the external factors which the insects are exposed to in the different literature sources, inevitably varying in the results obtained (Payne *et al*., 2016).

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Table 4.4 Amino acid values (mg.g^{-1} protein) of South African staple food products (flour, bread and maize meal) expressed as the percentage contribution to the amino requirement for adults (older than 18 years).

	AA[a] (mg.g^{-1} protein)																			
	CYS		**HIS**		**ILE**		**LEU**		**LYS**		**MET**		**PHE + TYR**		**THR**		**TRP**		**VAL**	
	V[b]	%[c]	V[b]	%[c]	V[b]	%[c]	V[b]	%[c]	V[b]	%[c]	V[b]	%[c]	V[b]	%[c]	V[b]	%[c]	V[b]	%[c]	V[b]	%[c]
White bread flour[1]	3.43	57.17	3.14	20.93	4.32	14.40	7.78	13.19	2.31	5.13	1.78	11.13	15.17	39.92	3.39	14.74	1.28	21.33	4.83	12.38
Brown bread flour[1]	3.12	52.00	3.20	21.33	3.58	11.93	7.12	12.07	2.18	4.84	1.91	11.94	7.8	20.53	3.33	14.48	1.54	25.67	4.56	11.69
White bread[1]	3.15	52.50	2.17	14.47	2.88	9.60	5.28	8.95	1.6	3.56	1.44	9.00	6.01	15.82	2.34	10.17	1.02	17.00	3.31	8.49
Brown bread[1]	3.11	51.83	2.19	14.60	2.82	9.40	5.09	8.63	1.83	4.07	1.13	7.06	5.81	15.29	2.31	10.04	1.10	18.33	3.26	8.36
Super MM[d; 2]	0	0	2.10	14.00	2.90	9.67	11.00	18.64	1.60	3.56	1.70	10.63	4.00	10.53	2.60	11.30	0	0	4.10	10.51
Special MM[d; 2]	0	0	2.20	14.67	3.00	10.00	11.20	18.98	1.80	4.00	1.80	11.25	4.20	11.05	2.70	11.74	0	0	4.30	11.03
Sifted MM[d; 2]	0	0	2.30	15.33	3.20	10.67	11.50	19.49	2.00	4.44	1.90	11.88	4.40	11.58	2.80	12.17	0	0	4.40	11.28
Unsifted MM[d; 2]	0	0	2.40	16.00	3.30	11.00	11.60	19.66	2.10	4.67	2.00	12.50	4.50	11.84	3.00	13.04	0	0	4.60	11.79

[a] Abbreviations of AA's: **CYS**: Cysteine; **HIS**: Histidine; **ILE**: Isoleucine; **LEU**: Leucine; **LYS**: Lysine; **MET**: Methionine; **PHE**: Phenylalanine; **TYR**: Tyrosine; **THR**: Threonine; **TRP**: Tryptophan and **VAL**: Valine. [b] V: Value; [c] %: Value expressed as percentage contribution to the AA requirement as stipulated in Table 3.2; [d] MM: Maize meal.

References: [1]Danster *et al.*, 2008; [2]Medical Research Council, 2002.

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Table 4.5 Amino acid values (mg.g^{-1} protein) of edible insects consumed in South Africa expressed as a percentage contribution to the amino requirement for adults (older than 18 years). Amino acid values (mg. g^{-1} protein) are based on protein values in Table 3.4.

	AA[a] (mg.g^{-1} protein)									
	CYS	**HIS**	**ILE**	**LEU**	**LYS**	**MET**	**PHE + TYR**	**THR**	**TRP**	**VAL**
	M ± SD[b] (%[c])	**M ± SD[b] (%[c])**	**M ± SD[b] (%[c])**	**M ± SD[b] (%[c])**	**M ± SD[b] (%[c])**	**M ± SD[b] (%[c])**	**M ± SD[b] (%[c])**	**M ± SD[b] (%[c])**	**M ± SD[b] (%[c])**	**M ± SD[b] (%[c])**
Sterconera orissa[1]	-	13.60 (90.65)	22.17 (73.90)	35.34 (59.90)	29.52 (65.61)	6.08 (38.03)	29.42 (77.42)	18.52 (80.52)	5.56 (92.59)	21.32 (54.67)
Oryctes monoceros[2]	20.80 (346.67)	28.80 (192.00)	30.40 (101.33)	63.00 (106.78)	28.30 (62.89)	20.80 (130.00)	68.5 (180.26)	29.00 (126.09)	21.00 (350.00)	26.40 (67.69)
Tenebrio molitor [3 - 11]	12.61 ± 21.19 (210.17)	40.54 ± 28.61 (270.27)	50.36 ± 20.44 (167.87)	70.89 ± 36.91 (120.15)	56.79 ± 22.46 (126.20)	9.19 ± 6.55 (57.44)	84.38 ± 40.32 (22.05)	41.48 ± 14.81 (180.35)	7.02 ± 9.26 (117.00)	63.70 ± 19.22 (163.33)
Encosternum delegorguei[12]	-	-	23.60 (78.67)	29.80 (50.51)	24.10 (53.56)	11.40 (71.25)	23 (60.53)	23.30 (101.30)	4.50 (75.00)	-
Apis mellifera[13 - 15]	12.97 ± 7.22 (216.17)	26.24 ± 5.64 (174.93)	45.08 ± 4.17 (150.27)	60.77 ± 17.45 (103.00)	60.42 ± 2.00 (134.27)	23.06 ± 2.49 (144.13)	79.25 ± 34.07 (208.55)	40.22 ± 6.25 (174.87)	8.29 ± 1.85 (138.17)	54 ± 3.89 (138.46)
Macrotermes falciger[16,17]	3.01 (50.17)	45.13 ± 22.81 (300.87)	41.09 ± 3.67 (136.97)	72.02 ± 1.45 (122.07)	75.00 ± 15.55 (166.67)	16.98 ± 2.80 (106.13)	115.78 ± 13.12 (304.68)	41.54 ± 5.00 (180.61)	8.09 (134.83)	50.83 ± 0.95 (130.33)
Cirina forda[18,19]	9.64 ± 4.29 (160.67)	22.55 ± 2.47 (150.33)	36.65 ± 0.21 (122.17)	65.09 ± 8.46 (110.32)	51.64 ± 8.11 (114.76)	14.69 ± 12.00 (91.75)	82.3 ± 6.08 (216.58)	44.85 ± 9.97 (195.00)	18.40 (306.67)	44.52 ± 9.17 (114.15)
Gonimbrasia belina[20, 21]	19.65 ± 4.74 (327.50)	30.15 ± 1.34 (201.00)	31.15 ± 1.34 (103.83)	60.55 ± 14.50 (102.63)	44.35 ± 4.03 (98.56)	18.55 ± 5.02 (115.94)	89.25 ± 33.02 (234.87)	59.45 ± 19.02 (258.48)	20.60 ± 13.58 (343.33)	36.20 ± 6.36 (92.82)
Imbrasia ertli [22]	13.40 (223.33)	-	36.00 (120.00)	36.70 (62.20)	39.30 (87.33)	15.80 (98.75)	30.6 (80.53)	40.50 (176.09)	8.10 (135.00)	41.90 (107.44)
Imbrasia epimethea [23]	18.70 (311.67)	19.70 (131.33)	28.60 (95.33)	81.00 (137.29)	74.20 (164.89)	22.40 (140.00)	140 (368.42)	48.00 (208.70)	16.00 (266.67)	102.00 (261.54)

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Table 4.5 (Continued)

	AA[a] (mg.g^{-1} protein)									
	CYS	**HIS**	**ILE**	**LEU**	**LYS**	**MET**	**PHE + TYR**	**THR**	**TRP**	**VAL**
	M ± SD[b] (%[c])	**M ± SD[b] (%[c])**	**M ± SD[b] (%[c])**	**M ± SD[b] (% [c])**	**M ± SD[b] (% [c])**	**M ± SD[b] (% [c])**	**M ± SD[b] (% [c])**	**M ± SD[b] (% [c])**	**M ± SD[b] (% [c])**	**M ± SD[b] (% [c])**
Gynanisa maja[17]	3.93 (65.57)	45.24 (301.62)	33.62 (112.06)	48.64 (82.44)	71.89 (159.75)	14.66 (91.65)	35.59 (93.66)	40.41 (175.72)	13.41 (223.53)	37.37 (95.83)
Locusta migratoria[24]	-	14.00 (93.33)	25.00 (83.33)	46.00 (77.97)	29.00 (64.44)	-	-	19.00 (82.61)	4.00 (66.67)	41.00 (105.13)
Schistocerca gregaria[11]	3.60 (60.00)	20.60 (137.33)	28.20 (94.00)	77.70 (131.69)	35.10 (78.00)	8.20 (51.25)	51.80 (136.32)	35.50 (154.35)	-	56.60 (145.13)
Ruspolia differens[17, 25]	3.27 ± 2.41 (54.50)	62.36 ± 51.68 (415.73)	53.21 ± 7.52 (177.37)	76.27 ± 23.18 (129.27)	91.32 ± 52.90 (202.93)	8.52 ± 1.58 (53.25)	101.54 ± 19.42 (267.21)	53.32 ± 15.30 (231.83)	4.72 ± 5.72 (78.67)	51.10 ± 20.26 (131.03)
Zonocerus variegatus[26]	6.50 (108.33)	39.20 (261.33)	36.70 (122.33)	50.60 (85.76)	48.40 (107.56)	18.90 (118.13)	55.80 (146.84)	30.70 (133.48)	-	35.40 (90.77)
Hermetia illucens[6, 27 - 31]	10.28 ± 11.47 (171.33)	45.02 ± 27.94 (300.13)	40.88 ± 3.29 (136.27)	69.86 ± 4.73 (118.41)	56.79 ± 10.01 (126.20)	21.63 ± 12.17 (135.19)	100.29 ± 16.79 (263.92)	39.94 ± 3.71 (173.65)	14.07 ± 4.60 (234.50)	59.41 ± 9.87 (152.33)

[a] Abbreviations of AA's: **CYS**: Cysteine; **HIS**: Histidine; **ILE**: Isoleucine; **LEU**: Leucine; **LYS**: Lysine; **MET**: Methionine; **PHE**: Phenylalanine; **TYR**: Tyrosine; **THR**: Threonine; **TRP**: Tryptophan and **VAL**: Valine. [b] M ± SD: Mean ± Standard deviation; [c] %: Value expressed as percentage requirement as stipulated in Table 3.2.

References: [1]Shadung *et al*., 2012; [2]Ifie & Emeruwa, 2011; [3]Bosch *et al*., 2014; [4]Ghosh *et al*., 2017; [5]Hopley, 2016; [6]Janssen *et al*., 2017; [7]Ravzanaadii *et al*., 2012; [8]Xingqian *et al*., 1998; [9]Yi, 2015; [10]Zhao *et al*., 2016; [11]Zielińska *et al*., 2015; [12]Teffo *et al*., 2007; [13]Bednářová *et al*., 2013; [14]Finke, 2005; [15]Ghosh *et al*., 2016; [16]Phelps *et al*., 1975; [17]Siulapwa *et al*., 2014; [18]Igbabul *et al*., 2014; [19]Inje *et al*., 2018; [20]Ekpo, 2011; [21]Ohiokpehai, 2006; [22]Santos Oliveira *et al*., 1976; [23]Kodondi *et al*., 1987; [24]Purscke *et al*., 2018; [25]Fombong *et al*., 2017; [26]Adeyeye, 2005; [27]Caligiani *et al*., 2018; [28]Finke, 2013; [29]Liland *et al*., 2017; [30]Liu *et al*., 2017; [31]Spranghers *et al*., 2018

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The AA values of staple food products (Table 4.4) correlates with studies identifying lysine as the first limiting AA, with unfortified white bread flour containing the highest lysine content of 2.31 $mg.g^{-1}$ protein (resulting in 5.13% contribution to daily AA requirement per 100g product) (Bukkens, 1997; Dewettinck *et al*., 2008; Awika, 2011). Considering the aforementioned, it would be unfeasible to rely on unfortified staple food products to meet the daily lysine requirements due to the significant amount of product that would need to be consumed.

The overall AA content of edible insects (Table 4.5) corresponded with findings that the majority of insects have favourable threonine and lysine content but are generally lower in methionine (Igbabul *et al*., 2014). Lysine is abundant in edible insects frequently consumed in South Africa (Table 4.5). *Ruspolia differens* can assist in meeting the daily AA requirement, contributing over 200% of the daily lysine requirement (Siulapwa *et al*., 2014; Fombong *et al*., 2017). Furthermore, the high lysine content (44.35 $mg.g^{-1}$ protein) of *Gonimbrasia belina* and the tryptophan content supplying over three times the daily requirement correlates with literature indicating the overall favourable AA content in meeting the human requirements (Ohiokpehai, 2006; Ekpo, 2011; Payne *et al*., 2016). Little evidence has however been published on the dangers of lysine overconsumption in humans (Garlick, 2004). A study by Pencharz *et al*. (2008) accentuates that in certain instances, AA supplementation which exceeds the requirements, can be viewed as beneficial, especially for population groups' whose staple diet consists of maize and wheat. On the other hand, although most individuals tolerate higher tryptophan consumption relatively well, a minority of people may experience certain side effects when increasing their tryptophan consumption. These side effects include nausea, headaches, drowsiness and even slightly elevate the risk of cardiac dysfunctions (Kapalka, 2010). Uncertainty however still exists on the maximum levels which are safe for consumption (Pencharz *et al*., 2018).

An example of another edible insect with a significant AA content, is *Imbrasia epimethea* who has higher AA values than *Tenebrio molitor*, except for histidine and isoleucine. This is in accordance to a study by Lautenschläger *et al*. (2017), indicating *Imbrasia epimethea*'s favourable AA content. Furthermore, considering the advantage of no significant changes in nutritional content after processing, *Imbrasia epimethea* has the potential to act as complimentary food source to staple food products or alternative to animal-based sources (Lautenschläger *et al*., 2017).

In Table 4.4, the tryptophan and cysteine content of maize meal are indicated as zero. This corresponds with other literature sources which indicate that tryptophan is often classified as a limiting AA in grain products (Bukkens, 1997; Vasal, 2000). The degermination process of maize has been postulated as a possible reason for the low tryptophan content (WHO, 2000). Caution should be given to heat treatment applied as it can further decrease tyrosine's biovavailability (Delgado-Andrade, 2006). Bukkens (1997) mentioned the possibility of termites acting as a supplementary source alongside staple foods, due to the favourable tryptophan content. *Macrotermes falciger* (roasted winged termites), forming part of the termitidae family, corresponds to the aforementioned

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statement in that it exceeds the daily tryptophan requirement (6 mg.g^{-1} protein) in providing 8.09 mg.g^{-1} protein (Siulapwa *et al*., 2014). *Locusta migratoria*, has the lowest tryptophan content, but still contributes to reaching more than 60% of the daily requirement (Purscke *et al*., 2018). Table 4.5 therefore accentuates the potential of all edible insects consumed in South Africa as listed, to contribute to at least half of the daily tryptophan requirement. Consideration should however be given to the method identified for tryptophan quantification as acid hydrolysis can degrade the tryptophan content (Delgado-Andrade, 2006). It is therefore proposed to identify a standardised method for tryptophan quantification (Molnár-Perl, 1999).

Threonine is another example of a limiting AA in certain grain products (Prasanna *et al*., 2001). The favourable threonine content of edible insects consumed in South Africa, further supports and strengthens the evidence for insects as significant nutrient sources. All insects contributed to more than 80% of the daily requirement for threonine. *Gonimbrasia belina*, *Ruspolia differens* and *Imbrasia epimethea* which contains 59.45; 53.32 and 48.00 mg threonine.g^{-1} protein respectively (Kodondi *et al*., 1987; Ohiokpehai, 2006; Ekpo, 2011; Siulapwa *et al*., 2014; Fombong *et al*., 2017). The threonine content of staple food products and edible insects are almost incomparable. *Gomimbrasia belina* contains 78% more threonine than unsifted maize meal.

In line with previous literature published, edible insects consumed in South Africa could potentially contribute to the overall AA requirements of humans (Bukkens, 2005; Finke, 2005; Van Huis, 2013) From considering the before mentioned information and in viewing Table 4.5, it becomes clear that certain insect species stand out from the rest, regarding their favourable AA content. These insect species include *Ruspolia differens*, *Imbrasia epimethea*, *Macrotermes falciger* and *Gonimbrasia belina*. A number of beforementioned insects identified to contain significant AA contents, corresponds with the insects recognised for their favourable micronutrient content (section 4.2). *Ruspolia differens* and *Gonimbrasia belina* are edible insects consumed in South Africa which therefore not only contains favourable micronutrient content but has the potential to assist in meeting the AA requirements of adult as well. However, careful consideration must be given to the promotion of *Gonimbrasia belina* (mopane worms) harvesting, as numbers decreased immensely due to years of extensive gathering practises (Thomas, 2013).

In conclusion, through the drawing of parallels between the AA content of the edible insects identified above and the limiting AA's in staple food products, convincing evidence of these insects supplementing staple food products' AA content arises.

4.4 Protein content of edible insects

4.4.1 Kjeldahl and Dumas methods

Jonas-Levi & Martinez (2017) highlighted the paramount importance that the specific quantification method will play in the results obtained for edible insects' protein content. Throughout Table 4.6 it is

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evident that most studies utilised the Kjeldahl method as primary protein quantification method. Although various other papers have recommended alternative protein determination methods, especially AA analysis, to obtain more accurate results, Kjeldahl is still the preferred method of choice (Hall & Schönfeldt, 2013; Jonas-Levi & Martinez, 2017).

In Table 4.5, the Dumas method [D[p] (N x 6.25)[c]] was selected as the protein determination method for a sample obtained in the United States (Tinder *et al*., 2017). Comparable results to the aforementioned sample's protein content (42.88% w/w) were found by another sample obtained in the United states (42.10% w/w) and the Netherlands (42.14% w/w) (Makkar *et al*., 2014; Oonincx, 2015). As indicated, the latter United States sample (Makkar *et al*., 2014), determined through the Kjeldahl method, however had a slightly lower protein content than that of the Dumas method's sample (Tinder *et al*., 2017). This is in correspondence with research indicating the Dumas method's ability to measure a higher nitrogen content than Kjeldahl, inevitably resulting in a higher crude protein content (Mariotti *et al*., 2008; Sáez-Plaza *et al*., 2013; Müller, 2017).

4.6.2 Kjeldahl method modifications

4.6.2.1 Micro- and macro-Kjeldahl

Two respective studies which determined the protein content of *Macrotermes falciger*, did so with the aid of a micro- and macro-Kjeldahl method (Siulapwa *et al*., 2014; Chulu, 2015). The macro-Kjeldahl method yielded almost double the protein content (43.26% w/w) than that of the micro-Kjeldahl (23.10% w/w) (Siulapwa *et al*., 2014; Chulu, 2015). Differences in the macro- and micro Kjeldahl method can be contributed to the difference in sample size utilised and therefore the protein content obtained (Sáez-Plaza *et al*., 2013). Due to the variation in results, it is not possible to draw any definite conclusions on the suitability of *Macrotermes falciger* as a significant protein source. As only speculation is possible, in the case of macro-Kjeldahl method providing the more accurate protein content, *Macrotermes falciger* will be deemed a more favourable protein source. If the micro-Kjeldahl method's protein content is closer to the true protein content, inevitably a larger amount of *M. falciger* will need to be consumed. This will ultimately lead to questioning the significance of *M. falciger* as protein source. The micro- and macro-Kjeldahl only focused on one species, but the principal is the same for all species and methods. Standardisation of methods is necessary to truly draw comparisons and conclusions on the protein content of various insects.

4.6.2.2 Spectrometric and colorimetric methods

In the case of *Oryctes Monoceros*, the protein content for one of the samples was determined with a modified Kjeldahl [Modified K][j] method in which spectrophotometric methods were used (Edijala *et al*.,2009). When compared with the general Kjeldahl method (36.45%w/w), the modified Kjeldahl method yielded a lower (25.97% w/w) protein content. Potential differences in results of modified and standard Kjeldahl methods have previously been reported (Kirk & Sawyer, 1991).

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Table 4.6 Compilation of edible insect species, the respective preparation method and quantification method employed to determine the protein content.

Preparation methods
[a]**OD**: Oven-dried; [d]**FD**: freeze dried; [e]**C**: Cooked; [f]**F**: Fried; [g]**B**: Boiled; [h]**SD**: Sun-dried; [k]**DR**: Dried; [o]**VC**: Vacuum cooked; [r]**PF**: Pan-fried; [s]**OC**: Oven cooked; [t]**BD**: Blast dried; [w]**R**: Roasted; [¥]**AD**: Air-dried
Quantification methods
[b]**K**: Kjeldahl method; [c]**(N x 6.25):** Nitrogen-to-protein conversion factor of 6.25; [j]**Modified K**: Modified Kjeldahl, protein spectrophotometrically determined;; [l]**CP**: Crude Protein; [m]**Modified K**: Modified Kjeldahl, protein content determined with Kjeltec analyser; [n]**Micro-K**: Micro-Kjeldahl; ; [p]**D**: Dumas method; [q]**(N x 4.76)**: Nitrogen-to-protein conversion factor of 4.76; [u]**Semimicro-K:** Semimicro-Kjeldahl method; [v]**AA analysis (HPLC)**: Amino acid analysis with HPLC;; [x]**Macro-K**: Macro-Kjeldahl method; [y]**AA analysis (-trp)**: Amino acid analysis, excluding tryptophan; [z]**Modified K**: Kjeldahl Analysed with Tru-Spec Protein Analyser ; [Δ]**N**: Total Nitrogen: Sample N content – N content in chitin; [ß]**Modified K**: Kjeldahl William colorimetric method; [ɸ]**(N x 6.61)**: Nitrogen-to-protein conversion factor of 6.61; [π]**N:** Nitrogen analysed with CHN analyser;
General
[i]**NS**: Not Specified; *****Bred in captivity

Species	Geographical area	Life stage	Preparation method	Quantification method	Protein (%w/w)
Sternocera orissa	Limpopo[1]	Adult[1]	[OD[a] (66°C for 24h)][1]	[K[b] (Nx6.25)[c]][1]	63.00[1]
	Limpopo[1]	Adult[1]	[FD[d] (55°C for 24h)][1]	[K[b] (Nx6.25)[c]][1]	22.51[1]
	Limpopo[1]	Adult[1]	(C[e], F[f])[1]	[K[b] (Nx6.25)[c]][1]	31.19[1]
Oryctes boas	Nigeria[2]	Larvae[2]	[OD][a, 2]	[K[b] (NS)[i]][2]	26.00[2]
	Nigeria[3]	Larvae[3]	[B[g] (15 min); SD[h] (21 days)][3]	[NS][i, 3]	55.82[3]
Oryctes monoceros	Nigeria[4]	Larvae[4]	[OD][a, 4]	[Modified K][j,4]	25.97[4]
	Nigeria[5]	Larvae[5]	[OD][a, 5]	[K[b] (NS)[i]][5]	36.45[5]
Oryctes owariensis	Côte d'Ivoire[6]	Larvae[6]	[OD[a] (65°C for 72h)][6]	[K[b] (Nx6.25)[c]][6]	50.64[6]
Tenebrio molitor	Indonesia[7]	Larvae[7]	[D[k] (100°C)][7]	[K[b] (N[Δ] x 6.25)[c]][7]	52.00[7]
	Brazil[8]*	Larvae[8]	[OD[a] (45°C for 48h)][8]	[K[b] (N x 6.25)[c]][8]	47.45[8]
	Netherlands[9]*	Larvae[9]	[D[k] (50°C for 96 h)][9]	[CP[l] (N x6.25)[c]][9]	53.21[9]
	Netherlands[10]*	Larvae[10]	[FD][d, 10]	[K[b] (Nx6.25)[c]][10]	52.00[10]
	[11]*	Larvae[11]	[F][f,11]	[K[b] (N x 6.25)[c]][11]	53.71[11]
	Canada[12]*	Larvae[12]	[FD][d, 12]	[Modified K][m, 12]	66.09[12]
	Korea[13]*	Larvae[13]	[FD][d, 13]	[K[b] (N x 6.25)[c]][13]	53.22[13]
	South Africa[14]*	Larvae[14]	[OD[a] (65°C for 72h)][14]	[CP[l] (N x 6.25)[c]][14]	50.16[14]
	Korea[15]	Larvae[15]	[OD[a] (105°C for 4h)][15]	[Micro-K (NS)][n,15]	49.80[15]
	Indonesia[16]	Larvae[16]	[OD[a] (60 - 70°C for 12-24h)][16]	[K[b] (NS)[i]][16]	38.30[16]
	Belgium[17]*	Larvae[17]	[VC[o] (74°C for 60min)][17]	[D[p] (N x 4.76)[q]][17]	42.60[17]
	Belgium[17]*	Larvae[17]	[PF[r] (1 min in olive oil)][17]	[D[p] (N x 4.76)[q]][17]	26.90[17]
	Belgium[17]*	Larvae[17]	[B[g] (100°C for 1 min)][17]	[D[p] (N x 4.76)[q]][17]	43.90[17]

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Table 4.6 (Continued)

Species	Geographical area	Life stage	Preparation method	Quantification method	Protein (%w/w)
	Belgium[17]*	Larvae[17]	[OC[s] (70°C for 30min)][17]	[D[p] (N x 476)[q]].[17]	43.40[17]
	Netherlands[18]*	Larvae[18]	-	[CP[l] (N x 6.25)[c]][18]	42.00[18]
	Netherlands[19]*	Larvae[19]	[DR][k, 19]	[K[b] (N x 6.25)[c]][19]	49.05[19]
	Netherlands[19]*	Larvae[19]	[DR][k, 19]	[K[b] (N x 6.25)[c]][19]	49.53[19]
	Belgium[19]*	Larvae[19]	[DR][k, 19]	[K[b] (N x 6.25)[c]][19]	53.97[9]
	Korea[20]*	Larvae[20]	-	[Modified K[m] (NS)[i]][20]	46.44[20]
	China[21]	Larvae[21]	[BD[t] (80 - 90°C)][21]	[Semimicro-K[u] (N x 6.25)[c]][21]	47.40[21]
	Poland[22]*	Larvae[22]	[B[g] (100°C for 10 min) / OD[a] (150°C for 10min)][22]	[K[b] (Nx 6.25)[c]][22]	52.35[22]
	Sweden[23]*	Larvae[23]	[FD][d, 23]	[Modified K[m] (N x 6.25)[c]][23]	51.50[23]
Encosternum delegorguei	Zimbabwe[24]	Adult[24]	[C[e] (3 min)][24]	[K[b] (N x 6.25)[c]][24]	33.20[24]
	Zimbabwe[25]	Adult[25]	[C[e] (3 min); OD[a] (60°C)][25]	[K[b] (NS)[i]][25]	37.45[25]
	Limpopo[26]	Adult[26]	[SD][h, 26]	[AA analysis (HPLC)[v] (N x 6.25)[c]][26]	35.20[26]
Carebara vidua	Zimbabwe[25]	Adult[25]	[OD[a] (60°C)][25]	[K[b] (NS)[i]][25]	43.60[25]
	Kenya[27]	Adult[27]	[F][f, 27]	[NS][l, 27]	40.83[27]
Apis mellifera	Nigeria[2]	eggs, larvae, pupae[2]	[OD][a, 2]	[K[b] (NS)[i]][2]	21.00[2]
	Czech Republic[28]*	larvae, pupae[28]	[F][f, 28]	[Modified K][j, 28]	54.38[28]
	United States[29]*	larvae, pupae[29]	[F][f, 29]	[CP[l] (N x 6.25)[c]][29]	40.52[29]
	Korea[30]*	larvae, pupae[30]	[OD][a,30]	[K[b] (N x 6.25)[c]][30]	40.70[30]
Odontotermes badius	Kenya[31]	Alate[31]	[F][f, 31]	[CP[l] (NS)[i]][31]	42.52[31]
Macrotermes falciger	Zambia[32]	Alates[32]	[R[w] (10min)][32]	[Micro-K (NS)][n, 32]	23.10[32]
	Zimbabwe[33]	Alates[33]	[VC[o] (60°C)][33]	[K[b] (NS)[i]][33]	41.80[33]
	Zambia[34]	Alate[34]	-	[Macro-K[x] (N x 6.25)[c]][34]	43.26[34]
Trinervitermes trinervoides	South Africa[36]	Adult[36]	[OD[a] (70°C)][36]	[CP[l] (N x 6.25)[c]][36]	46.00[36]

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Table 4.6 (Continued)

Species	Geographical area	Life stage	Preparation method	Quantification method	Protein (%w/w)
Macrotermes natalensis	Nigeria[2]	Alate[2]	[OD][a, 2]	[K[b] (NS)[i]][2]	22.10[2]
	Zimbabwe[25]	Alate[25]	[OD[a] (60°C)][25]	[K[b] (NS)[i]][25]	37.10[25]
	Nigeria[35]	Alate[35]	[OD[a] (45°C)][35]	[NS][i, 35]	65.62[35]
Cirina forda	Nigeria[35]	Larva[35]	[OD[a] (45°C)][35]	[NS][i, 35]	74.35[35]
	Nigeria[36]	Larvae[36]	[OD[a] (60°C for 72h)][36]	[NS][i, 36]	33.12[36]
	Nigeria[37]	Larvae[37]	[D][k, 37]	[CP[l] (NS)[i]][37]	55.44[37]
	Nigeria[38]	Larva[38]	[D][k, 38]	[NS][i, 38]	48.65[38]
	Nigeria[39]	Larva[39]	[OD[a] (40°C for 24h)][39]	[Micro-K[n] (N x 6.25)[c]][39]	55.50[39]
	Zambia[40]	Larva[40]	-	[CP[l] (N x 6.25)[c]][40]	62.40[40]
	Nigeria[41]	Larvae[41]	[SD][h, 41]	[NS][i, 41]	20.00[41]
	Nigeria[42]	Larvae[42]	[SD[h] (72h)][42]	[Micro-K[n] (N x 6.25)[c]][42]	31.30[42]
Gonimbrasia belina	Zimbabwe[25]	Larvae[25]	[SD][h, 25]	[K[b] (NS)[i]][25]	55.40[25]
	Zimbabwe[40]	Larvae[40]	[DR][k, 40]	[CP[l] (N x 6.25)[c]][40]	52.70[40]
	South Africa[43]	Larvae[43]	[FD][d, 43]	[CP[l] (N x 6.25)[c]][43]	62.50[43]
	Nigeria[44]	-	-	[Modified K[ɤ] (NS)[i]][44]	54.26[44]
	Zimbabwe[45]	Larvae[45]	[VC[o] (25°C)][45]	[AA analysis[y] (-Trp)][45]	48.27[45]
	Botswana[46]	Larvae[46]	[B[g] (45 min)][46]	[K[b] (NS)[i]][46]	44.94[46]
	Botswana[46]	Larvae[46]	[R (5-7min)][46]	[K[b] (NS)[i]][46]	46.85[46]
	Maunatlala, Botswana[47]	Larvae[47]	[B[g] (30min), SD[h] (4h), OD[a] (105C for 48h)]	[K[b] (NS)[i]][47]	54.94[47]
	Moreomabele, Botswana[47]	Larvae[47]	[B[g] (30min), SD[h] (4h), OD[a] (105C for 48h)]	[K[b] (NS)[i]][47]	48.43[47]
	Sefophe, Botswana[47]	Larvae[47]	[B[g] (30min), SD[h] (4h), OD[a] (105C for 48h)]	[K[b] (NS)[i]][47]	44.60[47]
Imbrasia ertli	Angola[49]	-	[C[e], R[w] or SD[h]][49]	[K[b] (N x 6.25)[c]][49]	48.66[49]
Imbrasia epimethea	Angola[50]	Larvae[50]	[B[g] (3 min), SD[h] (3 days); FD[d]][50]	[K[b] (N x 6.25)[c]][50]	70.80[50]
	Angola[50]	Larvae[51]	[SD][h, 51]	[CP[l] (N x 6.25)[c]][51]	62.50[51]
Gynanisa maja	Zimbabwe[25]	Larva[25]	[OD[a] (60°C)][25]	[K[b] (NS)[i]][25]	51.10[25]
	Zambia[34]	Larva[34]		[Macro-K[x] (NS)[i]][34]	55.92[34]
Bunaea alcinoë	Nigeria[35]	Larva[35]	[OD[a] (45°C)][35]	[NS][i, 35]	74.36[35]
	Nigeria[53]	Larvae[53]	[SD[h] (48h)][53]	[K[b] (NS)[i]][53]	44.23[53]

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Table 4.6 (Continued)

Species	Geographical area	Life stage	Preparation method	Quantification method	Protein (%w/w)
Ruspolia differens	Zambia[34]	Adult[34]	-	[Macro-K[x] (NS)[i]]	44.59[34]
	Uganda, Kenya[58]	Adult[58]	[OD[a] (60°C for 24h)][58]	[K[b] (N x 6.25)[c]][58]	47.70[58]
	Uganda, Kenya[58]	Adult[58]	[FD[d] (-50°C for 48h) and then at -55°C for 48h][58]	[K[b] (N x 6.25)[c]][58]	46.41[58]
	Kenya[59]	Adult[59]	[SD][h, 59]	[CP[l] (N x 6.25)[c]][59]	43.70[59]
Zonocerus variegatus	Nigeria[2]	Adult[2]	[OD][a, 2]	[K[b] (NS)[i]][2]	26.80[2]
	Nigeria[38]	Adult[38]	[DR][k, 38]	[NS][i, 38]	48.65[38]
	Nigeria[60]	Adult[60]	[B[g] (3min), OD[a] (60°C for 8h)][60]	[CP[l] (N x 6.25)[c]][60]	66.30[60]
	Nigeria[61]	Adult[61]	[OD[a] (55°C)][61]	[Micro-K[n] (N x 6.25)[c]][61]	54.90[61]
Hemijana variegata	Limpopo[54]	Larvae[54]	[OD[a] (60°C for 24h)][54]	[Modified K[z] (NS)[c]][54]	52.42[54]
	Limpopo[54]	Larvae[54]	[OD[a] (60°C for 48h)][54]	Modified K[z] (NS)[c]][54]	51.41[54]
	Limpopo[54]	Larvae[54]	[OD[a] (60°C for 72h)][54]	Modified K[z] (NS)[c]][54]	53.84[54]
Locustana migratoria	Czech Republic[28]*	Adult[28]	[F][28]	[Modified K[j]][28]	62.21[28]
	Spain[55]*	Adult[55]	[F][55]	[K[b] (N x 6.25)[c]][55]	58.50[55]
	Sudan[56]	Adult[56]	[OD at 45°C][56, a]	[CP[l] (NS)[i]][56]	50.42[56]
	[57]*	Adult[57]	[FD[57, c]	[K[b] (N x 6.25)[c]][57]	59.02[57]
Schistocerca gregaria	Poland[22]*	Adult[22]	[B[g] (10 min at 100°C) or OD[a] (50°C for 10min)][22]	[K[b] (N x 6.25)[c]][22]	76.00[22]
Hermetia illucens	Netherlands[18]*	Larvae[18]		[CP[l] (N x 6.25)[c]][18]	42.14[18]
	Italy[62]	Larvae[62]	[F][f, 62]	[K[b] (N x 6.61)[ɸ]][62]	32.00[62]
	United States[63]*	Larvae[63]	[F][f, 63]	[CP[l] (N x 6.25)[c]][63]	17.50[63]
	Denmark[64]*	Larvae[64]	[OD[a] (105°C for 24h)][64]	[CP[l] (N[π] x4.76)[q]][64]	37.00[64]
	China[65]*	Larvae[65]	[FD[d] (-20°C)][65]	[K[b] (NS)[i]][65]	47.20[65]
	United States[66]	Larvae[66]	[F[f], OD[a]][66]	[K[b] (NS)[i]] [66]	42.10[66]

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Table 4.6 (Continued)

Species	Geographical area	Life stage	Preparation method	Quantification method	Protein (%w/w)
	Kenya[67]*	Larvae[67]	[AD¥][67]	[Semimicro-K[u] (NS)[i]][67]	38.98[67]
	Belgium[68]*	Larvae[68]	[FD][d, 68]	[CP[l] (N x 6.25)[c]][68]	50.79[68]
	United States[69]*	Larvae[69]	[F[f], OD[a] (60°C)][69]	[D[p] (N x 6.25)[c]][69]	42.88[69]
	South Africa[70]	Larvae[70]	OD[a] (65°C for 72h)]	[CP[l] (N x 6.25)[c]][70]	45.60[70]

References: [1]Shadung *et al*., 2012; [2]Banjo *et al*., 2006; [3]Fasunwon *et al*., 2011; [4]Edijala *et al*.,2009; [5]Ifie & Emeruwa, 2011; [6]Assielou *et al*., 2015; [7] Adámková *et al*., 2017; [8]Alves *et al*., 2017; [9]Bjørge *et al*., 2018; [10]Bosch *et al*., 2014; [11]Frye & Calvert, 1989; [12]Ghaly & Alkoaik, 2009; [13]Ghosh *et al*., 2017; [14]Hopley, 2016; [15]Kim *et al*., 2017; [16]Kuntandi *et al*., 2018; [17]Megido *et al*., 2018; [18]Oonincx, 2015; [19]Osimani *et al*., 2017; [20]Ravzanaadii *et al*., 2012; [21]Xingqian *et al*., 1998; [22]Zielińska *et al*., 2015; [23]Zhao *et al*., 2016; [24]Musundire *et al*., 2016a; [25]Musundire *et al*., 2016b; [26]Teffo *et al*., 2007; [27]Ayieko *et al*., 2010; [28]Bednářová *et al*., 2013; [29]Finke, 2005; [30]Ghosh *et al*., 2016; [31]Kenji *et al*., 2010; [32]Chulu, 2015; [33]Phelps *et al*., 1975; [34]Siulapwa *et al*., 2014; [35]Agbidye *et al*., 2009; [36]Akinnawo & Ketiku, 2000; [37]Igbabul *et al*., 2014; [38]Ogunleye, 2006; [39]Omotoso, 2006; [40]Onigbinde & Adamolekun, 1998; [41]Osasona & Olaofe, 2010; [42]Paiko *et al*., 2014; [43]Dreyer & Wehmeyer, 1982; [44]Ekpo, 2011; [45]Glew *et al*., 1999; [46]Madibela *et al*.,2007; [47]Madibela *et al*., 2009; [48]Ohiokpehai, 2006; [49]Santos Oliveira *et al*., 1976; [50]Lautenschläger *et al*., 2017; [51]Williams, 2016; [52]Amadi *et al*., 2005; [53]Dauda *et al*., 2014; [54]Egan, 2013; [55]Barroso *et al*., 2014; [56]Mohamed, 2015; [57]Oonincx & van der Poel, 2010; [58]Fombong *et al*., 2017; [59]Kinyuru *et al*., 2010; [60]Ladeji *et al*., 2003; [61]Olaofe *et al*., 1998; [62]Caligiani *et al*., 2018; [63]Finke, 2013; [64]Gligorescu *et al*., 2018; [65]Liu *et al*., 2017; [66]Makkar *et al*., 2014; [67]Nyakeri *et al*., 2017; [68]Spranghers *et al*., 2018; [69]Tinder *et al*., 2017; [70]Bessa, 2016.

4.6.2.2 Spectrometric and colorimetric methods

In the case of *Oryctes Monoceros*, the protein content for one of the samples was determined with a modified Kjeldahl [Modified K][j] method in which spectrophotometric methods were used (Edijala *et al*.,2009). When compared with the general Kjeldahl method (36.45%w/w), the modified Kjeldahl method yielded a lower (25.97% w/w) protein content. Potential differences in results of modified and standard Kjeldahl methods have previously been reported (Kirk & Sawyer, 1991).

The modified Kjeldahl William colorimetric method used for the determination of nitrogen content in *Gonimbrasia belina* in Nigeria, yielded 54.26 % w/w crude protein when converted (Ekpo, 2011). This is comparable to the protein content of *Gonimbrasia belina* samples determined by the Kjeldahl method in two other geographical regions, Zimbabwe (55.40% w/w) and Botswana (54.94% w/w) (Madibela *et al*., 2009; Musundire *et al*., 2016b). In yielding similar results to the Kjeldahl method which is regarded as the standard method, the colorimetric method has the potential to be classified as a significant protein determination method (William, 1964; Sáez-Plaza *et al*., 2013).

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4.6.3 CNS Analyser

One study in Table 4.8 made use of the CNS analyser to determine the nitrogen content (N$^{\Gamma}$) (Gligorescu *et al*., 2018). The nitrogen of the *Hermetia illucens* sample was then multiplied with a Kp of 4.76. This is however the only study which utilised the 4.75 Kp as opposed to the standard 6.25 Kp. It is therefore assumed that in multiplying the recovered nitrogen with a higher Kp, will result in a higher crude protein content. Comparisons between the determination methods cannot be made due to the variance in Kp's.

4.6.4 Amino acid hydrolysis

In Table 4.8, the AA analysis was only utilised as a protein determination method in two of the insect species, namely *Encosternum delegorguei* (35.20% w/w) and *Gonimbrasia belina* (48.27% w/w) (Glew *et al*., 1999; Teffo *et al*., 2007). In both species, the Kjeldahl results (33.20 – 37.45% w/w for *Encosternum delegorguei* and 44.60 – 55.40% w/w for *Gonimbrasia belina*) compared favourably to the overall AA analysis results. Furthermore, as the Kjeldahl and AA analysis yielded comparable results, both confirmed the promising protein content of *Gonimbrasia belina* as accentuated through ample literature sources (Van Huis, 2013; Payne *et al*., 2016).

The overestimation of protein content by the Kjeldahl method as reported by previous studies, was not visible from Table 4.6 (Hall & Schönfeldt, 2013; Jonas-Levi & Martinez, 2017). No significant differences could be detected in the protein content of samples determined through the Kjeldahl method compared to those determined through AA hydrolysis. Due to the limited studies indicating AA hydrolysis as protein determination process in Table 4.6, it is not possible to draw conclusions on literature identifying AA hydrolysis as the superior choice, yielding more accurate results (Owusu-Apenten, 2002; Mæhre *et al*., 2018).

4.6.5 Kp adjustment

A sample of the *Hermetia illucens* larvae from Denmark utilised an alternative Kp of 4.76 and yielded a lower protein content of 37% w/w (Gligorescu *et al*., 2018). In lowering the Kp, it was therefore expected that the protein content of the sample will be lower than the Dumas method's sample (42.88% w/w), which utilised a 6.25 Kp (Tinder *et al*., 2017). From viewing the crude protein content of *Hermetia illucens* (Table 4.6), exceptions to the before mentioned statement is evident. Two other studies, which utilised higher Kp's (6.61 and 6.25) (Finke, 2013; Caligiani *et al*., 2018), revealed lower protein contents (32% w/w and 17.50% w/w) of *Hermetia illucens* than that obtained by the CNS analyser. Possible explanations for this occurrence includes different external factors and processing methods to which the *Hermetia illucens*' samples were exposed to, which could have potentially influenced the results.

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4.6.6 Chitin content consideration

One study determined the nitrogen content of the *Tenebrio molitor* sample by subtracting the chitin-nitrogen from the total nitrogen [K^b (N^Δ x 6.25)^c] (Adámková *et al*., 2017). The altered nitrogen content however yielded a similar protein result (52% w/w) to that of multiple other *T. molitor* samples determined through the Kjeldahl method with the same Kp of 6.25 (49.05 – 53.97% w/w) (Frye & Calvert, 1989; Bosch *et al*., 2014; Zielińska *et al*., 2015; Adámková *et al*., 2017; Ghosh *et al*., 2017; Osimani *et al*., 2017).

4.6.7 Processing method

Megido *et al*., 2018, indicated a less than ideal processing method in the specified study. The *Tenebrio molitor* samples were subjected to four processing methods, namely vacuum-cooked [VC^o (74°C for 60min)], pan fried [PF^r (1 min in olive oil)], boiled [B^g (100°C for 1 min)] and oven-cooked [OC^s (70°C for 30min)]. PF yielded the lowest protein content (26.90% w/w), whereas the other three methods had similar results, 42% w/w for VC, 43.9% w/w for B and 44.2% w/w for OC (Megido *et al*., 2018).

4.6.8 Concluding remarks

Through the exploration of edible insects consumed in South Africa's protein content, it is evident that the protein quantification method is not the only determining factor in the protein content obtained. The presence of additional factors, including geographical area, processing method and chitin content have the potential to influence results. It is therefore not possible to draw conclusions from the information available as to whether the Kjeldahl method does overestimate the protein content of edible insects or to propose possible alternative protein quantification methods.

4.7 Market accessibility and demand for edible insects

4.7.1 Edible insect market growth

When viewing Fig 4.1, an increase (33%) in edible insect market is projected between 2018 – 2022, when compared to every US$1 billion in the global meat market. Various reasons can be postulated for the increase in edible insect market. These reasons include increasing exposure to insect products, high population increase predicted in the African continent (already known for entomophagy), influence by celebrities' consumption habits, inclusion of convenience factor through online shopping and increased investment in the market (Tan *et al*., 2015; Verbeke, 2015; Lacey, 2016; Manuell, 2016).

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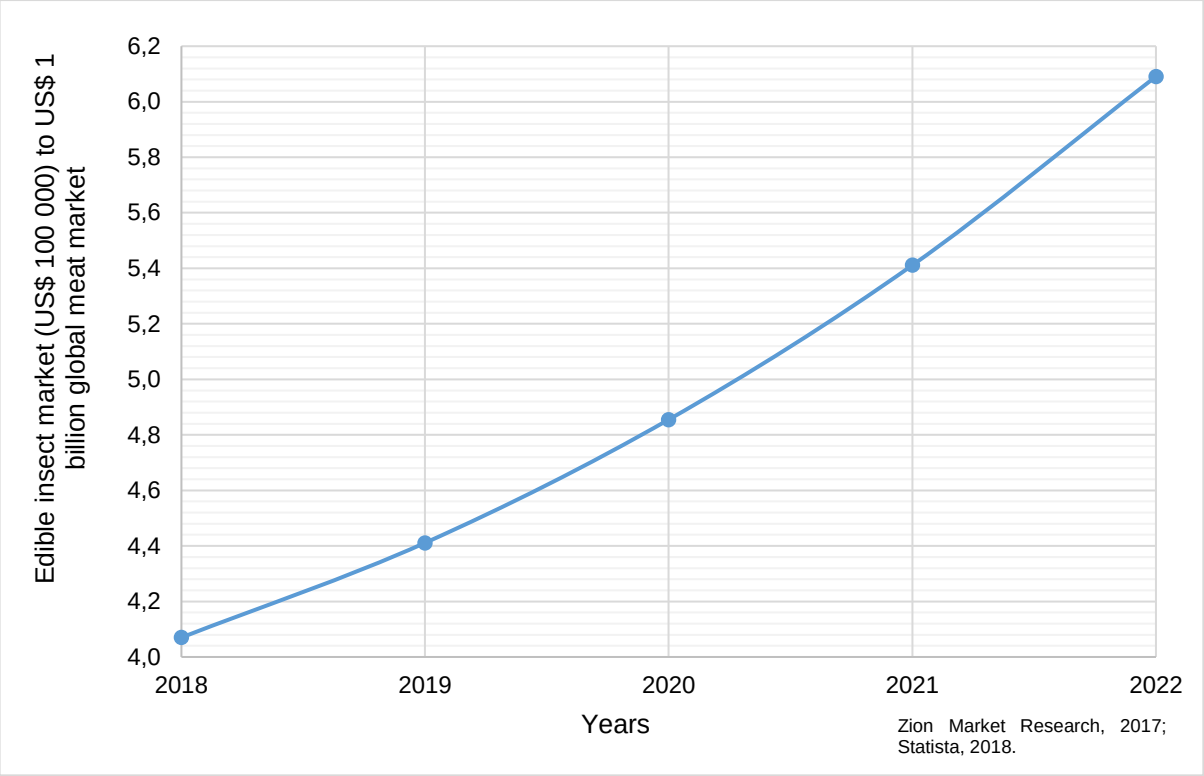

Figure 4.1 Value of global edible insect market (US$ 100 000) when compared to US$1 billion in global meat market between 2018 – 2022.

Even though the value of the edible insect market is improving, the global meat market is still the primary leader in protein-based sources, expected to be to worth more than US$ 1567 billion in 2022. A lack in standardised food safety systems of edible insects, the price and overall reluctance in accepting insects as food source, are major contributors to the diminutive increase in edible insect market compared to global meat market (Rumpold & Schlüter, 2013; Baker *et al*., 2016; Halloran *et al*., 2016; Schlup & Brunner, 2018). Various solutions, including supermarket involvement in standardising quality, selecting edible insect species target group are familiar with and including insects into well-known products have been suggested (Tan *et al*., 2015; Ayieko *et al*., 2016; Halloran *et al*., 2016). Currently, it is however not yet possible to conclude whether these proposed solutions can significantly influence the edible insect market.

4.7.3 Protein-based sources: price and protein comparison

Entomophagy has been labelled as superior to other animal-based food sources on multiple areas, including nutrient content, resource utilisation and decreased environmental pressure (Van Huis, 2013). A pivotal factor, namely price comparison between these sources are however often overlooked. Fig 4.2 and 4.3 illustrates the protein-price comparison.

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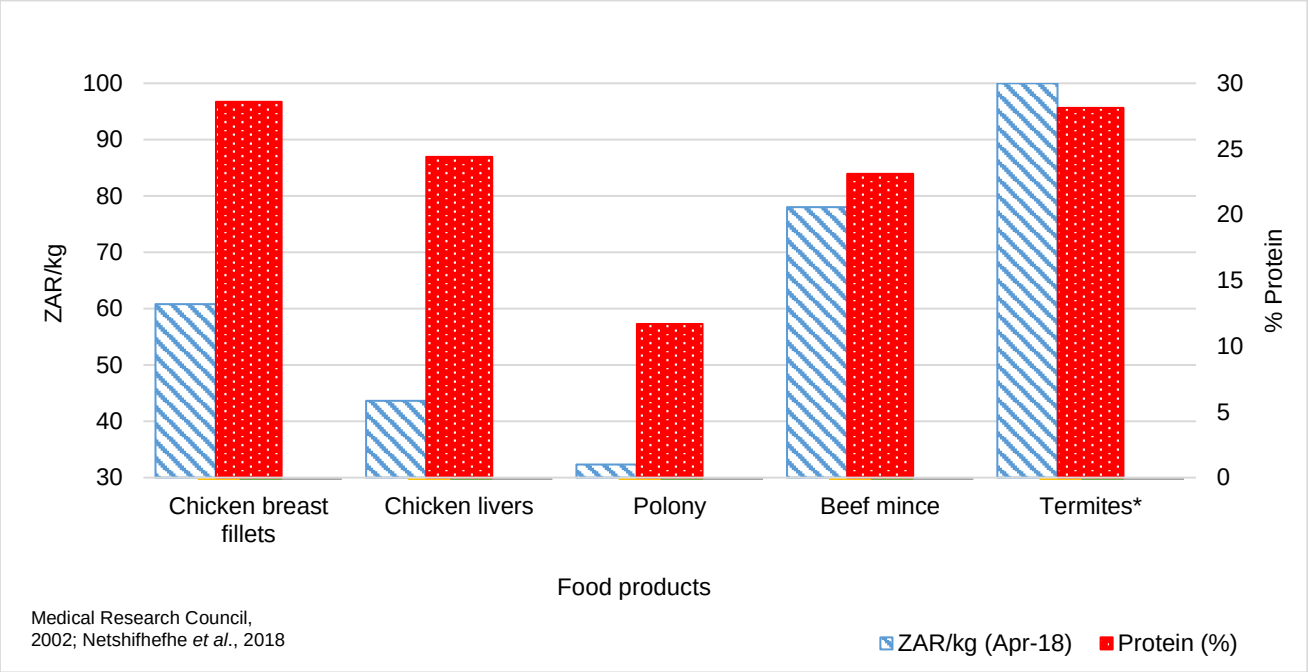

Figure 4.2 The price per kg in South African Rand (ZAR/kg) and percentage protein (%protein) of animal-based protein sources and termites.

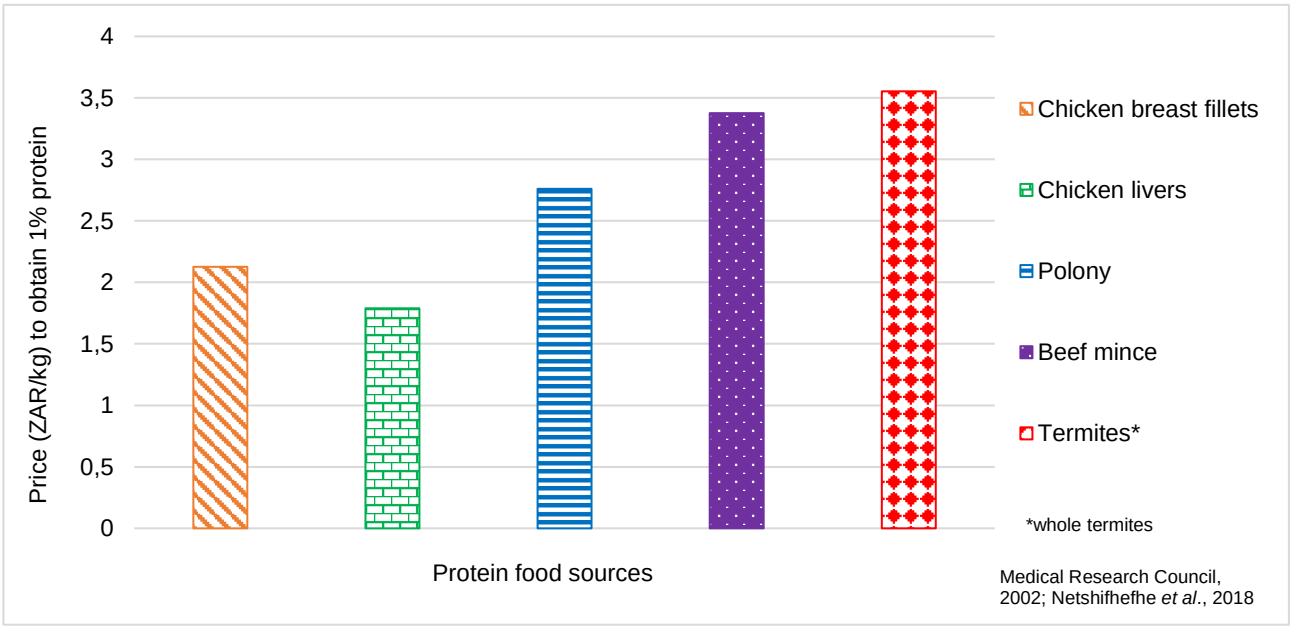

Figure 4.3 Price per kg in South African Rand (ZAR/kg) to obtain 1% protein of the respective protein source.

Currently, edible insects are expensive when compared to other animal-based sources. Even though the protein content of whole termites (28.14%) is in close range with chicken breast fillets (28.6%), the price of termites exceeds that of chicken by almost R40 per kg (Fig 4.2). Following whole termites, beef mince is the most expensive to obtain 1% protein. Polony is the least expensive food product in Fig 4.2, but also contains the least amount of protein. However, due to smaller difference between price (ZAR/kg) and percentage protein, chicken livers are also the cheapest form of protein to obtain 1% protein (Fig 4.3).

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The aforementioned information illustrates the high price of termites, which will inevitably affect its supply and demand. Refraining from purchasing edible insects due to the price, will exclude a wide proportion of consumers, especially low-income groups. Including the target group (those most likely to be affected by nutrient deficiencies), will require consistent collaboration in all areas of the value chain (Bessa *et al*. 2018). Upscaling edible insect production facilities and increased competitiveness in the market, can also assist in reaching the “availability” and “accessibility” domains of future edible insect food security (Barrett, 2010; De Schutter & Vanloqueren, 2011; Cortes Ortiz *et al*., 2016; Dobermann *et al*., 2017).

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Chapter 5: Conclusions and Recommendations

Currently it is clear that the nutritional programs implemented in South Africa, are not sufficient in ameliorating the nutritional deficiencies present. In conjunction with the increasing population, unsustainable and resource intensive production processes and alarming GHG emissions, it is clear that a drastically altered approach is needed. Through this research assignment, edible insects consumed in South Africa proved that they have the potential to contribute or fill this enormous nutritional gap, while adhering to the food and nutrition security dimensions.

Three main areas were investigated during this research assignment to meet the primary objective, which consisted of assessing the potential of edible insects frequently consumed in South Africa, in ameliorating South Africa's most prevalent nutrient deficiencies. The main objective of the research assignment was achieved through meeting the primary sub-objectives.

The most prevalent nutrient deficiencies in South Africa identified through various literature sources, includes iron, zinc, folate, vitamin A and iodine. Compared to the other deficiencies listed, zinc had the highest deficiency rate (49.30 – 73.90%), followed by mild iodine deficiency (41% for females and 34.90% males). Disparities and inconsistencies regarding the statistics were however noticeable. Factors including HIV/AIDS status, questioning the reliability of micronutrient determining methods, provision of additional micronutrient supplementation and exemption of mandatory usage of iodised salt during staple food production, can all influence the deficiency rates. Furthermore, a lack of micronutrient deficiency statistics, including folate deficiency rate was prevalent, especially for men. It was therefore concluded that even though staple food products are fortified, it is not sufficient to significantly reduce the deficiency rates in South Africa. The realisation became clear that alternative options needs to be explored to alleviate micronutrient deficiencies in South Africa.

In investigating the potential of edible insects as alternative food sources and the potential to contribute to meeting the micronutrient requirements, the primary sub-objective was met. Edible insect species frequently consumed in South Africa were identified and their micronutrient and amino acid content were obtained through secondary literature sources. As the research assignment mainly consisted of existing literature as a primary source of data collection, a limitation pertaining to this method is the dependency on the quality and quantity of secondary sources. In being dependent on current published literature, the possibility of finding incomplete or outdated data cannot be excluded.

Furthermore it became evident, through collecting data, that only a small proportion of the edible insects' nutritional content was actually determined in South Africa. The utilisation of data from the same edible insect species but from various other geographical areas and exposure to other external factors, could influence the result. This is evident through viewing the micronutrient and AA content as that the standard deviation often exceeded that of the mean values. As the number of nutrient values, which are included from other secondary sources, increases, the larger the likelihood of obtaining a higher standard deviation.

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In comparing the respective nutrient contents of edible insects and staple food products, it was possible to draw parallels between nutrients devoid in South African food products and insects rich in those specific nutrients. *Ruspolia differens* had the highest iron content (117.2 mg.100g^{-1} product) of the edible insect listed, whereas the highest iron content of unfortified staple food products was brown bread flour with 2.5 mg.100^{-1} product. During the research assignment a paucity in the iodine values available for staple food products and edible insects, except for *Hermetia illucens*, became clear. *Hermetia illucens* however only contributed 12.28% of the RDA of iodine per 100 g product consumed. Furthermore, except for *Carebara vidua* which contributes to 85.2% of the RDA females and 109.57% to males' RDA of vitamin A, the majority of selected insects were not significant sources of vitamin A. Consideration should therefore be given to the appropriateness of edible insects in supplementing staple food products to meet the RDA of vitamin A. Furthermore, *Sternocera orissa* can potentially supplement or compliment the low zinc content of staple food products (ranging between 0.58 - 1.90 mg.100g^{-1} product) as it provides more than ten times the RDA of zinc for men and eight times for females.

Insects have favourable amino acid contents, especially lysine, tryptophan and threonine, which are often classified as the limiting AA in staple food products. Methionine was however identified as the AA occurring in lower quantities in the majority of insects. The available AA data of the staple food products accentuates the limiting AA contents, with the highest lysine (2.31 mg.g^{-1} protein) and threonine content (3.39 mg.g^{-1} protein) occurring in white bread flour and a tryptophan content of 1.54 mg.g^{-1} protein in brown bread flour. *Ruspolia differens* and *Gonimbasia belina* were identified as significant sources of lysine, individually containing 91.32 mg and 44.35 mg lysine per gram protein. *Gonimbrasia belina* and *Macrotermes falciger* further contains favourable amounts of tryptophan (20.60 mg.g^{-1} protein and 8.09 mg.g^{-1} protein), which correlates to supplying 343.33% and 134.83% of the daily AA requirement. Lastly, *Gonimbrasia belina*, *Ruspolia differens* and *Imbrasia epimethea* indicated to contain favourable threonine amounts, resulting in 59.45; 53.32 and 48.00 mg threonine.g^{-1} protein respectively. Future recommendations can include the exploration of total amino acid levels and the ratio of amino acids relative to the lysine levels.

The exploration of edible insects and the potential to act as a supplementary or complimentary food source, revealed a promising future in ameliorating the most prevalent South African deficiencies. Various edible insects consumed in South Africa have the potential to contribute to more than half or even exceed the RDA of micronutrients or AA requirement. However, with concerns regarding the maximum micronutrient and AA levels deemed safe for consumption along with the potential to be more cost-effective, a smaller amount of certain insect species can be consumed and still meet half of the RDA for nutrients. These insects were identified as *Ruspolia differens*, *Sternocera orissa*, *Carebara vidua* and *Gonimbrasia belina*. For instance, *Sternocera orissa* has the ability to provide 50% of the RDA of men and women's zinc and iron content when 4.73 g and 10.63 g product are consumed respectively. Furthermore, in either consuming 32.26 g *Gonimbrasia belina*, 32.35 g *Ruspolia differens* or 58.67 g *Carebara vidua,* half of the RDA of folic

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acid will be met. Lastly, in consuming 32.35 g *Ruspolia differens*, males will receive four times the RDA of iron for females twice the amount of iron. *Ruspolia differens* and *Gonimbrasia belina* were further not only identified in containing favourable micronutrient amounts but also recognised for the significant potential in meeting the AA requirements of adults. The extensive harvesting process and drastic reduction in numbers of *Gonimbrasia belina* however raises concerns on further promoting the insect as significant micronutrient source.

The second part of the assignment consisted of evaluating the protein content of edible insects in South Africa and the concerns regarding the protein determination method of choice in providing reliable results. Through investigating the protein determination methods of edible insects, it became prevalent that the determination method is not the only factor influencing the protein content, but multiple other elements (geographical area, feed and processing method, chitin content and Kp adjustment) can also affect the results. Future recommendations regarding Kp is to establish all samples' nitrogen content through dividing the crude protein content with the specific Kp. Comparisons and discussions regarding the nitrogen content would be more beneficial if all insects' nitrogen values are available. The majority of the studies accessed in this research assignment, identified the Kjeldahl method as the protein determination method of choice, even though various alternative methods have been proposed.

Throughout the research assignment and in assessing the secondary sources' data, it became evident that a limited variety of determination methods were utilised. Similarly, the protein content of the edible insects was only established through AA analysis in two available studies (35.20% for *Encosternum delegorguei* and 48.27% w/w for *Gonimbrasia belina*). These results were however in correspondence with the Kjeldahl methods' results obtained for these species. AA analysis is often acknowledged as the protein determination method of choice, however as a result of limited data, it is not possible to effectively comment on this statement in the research assignment. In addition, due to the lack of alternative methods present to compare the Kjeldahl method to, it was not possible to draw any conclusions as to whether the Kjeldahl method leads to an overestimation of the protein content.

Furthermore, during the investigation of the effect of a reduction in Kp to 4.76, it was noticed that a reduced Kp did not automatically yield a lower crude protein content compared to when a higher Kp (6.61 and 6.25) was used. This was prevalent with a sample of *Hermetia illucens* (where a Kp of 4.76 was used), resulted in a protein content of 37% w/w, whereas when Kp's of 6.61 and 6.25 were used, lower protein contents of 32% w/w and 17.50% w/w were obtained. This can again be contributed to the presence of external factors affecting the results. Lastly, the potential influence of the processing method on the protein content of insects was investigated. Pan frying of *Tenebrio molitor* samples was accentuated as a less than ideal drying method. The pan fried Tenebrio molitor samples resulted in the lowest protein content when compared to vacuum-cooked, boiled and oven-

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cooked. It is suspected that the pan-frying method can reduce the protein digestibility of the sample, which can be attributed to the increased lipid content resulting in oxidation.

The third part of the research assignment consisted of investigating the viability of the edible insects in becoming a significant component of the global market. Findings project a 33% increase in the edible insect market between 2018 and 2022 when compared to every US$ 1 billion in the global meat market. The increase in the edible insect market is however still miniscule compared to the global meat market. A reluctance in accepting edible insects as a food source and the lack in standardised food safety systems in place are all postulated reasons for the slow growth. Various recommendations, including supermarket involvement in standardising food safety systems and incorporating insects into well-known products have been postulated, it is however currently not evident if these recommendations can truly promote growth of the edible insect market.

Lastly, the price of edible insects compared to other animal-protein sources was investigated. Even though whole termites have a protein content of 28.14%, comparable to that of chicken breast fillets (28.6%), termites cost almost R40 per kg more than chicken breast fillets. Termites was therefore deemed the most expensive food source to obtain 1% protein compared to chicken breast fillets, chicken livers, polony and beef mince. This raised questions as to how inclusive and accessible edible insects are to the majority of the population. The high cost of edible insects ultimately excludes a wide proportion of consumers, especially due to the process of urbanisation which reduces traditional harvesting patterns. Furthermore, the aforementioned population group (especially low-income consumers), are often suffering from nutritional deficiencies due to “accessibility” and “availability” constraints. A major shift is therefore needed from being classified as merely a niche product for high-income consumers or a food staple for local harvesters to being accessible and available for the majority of the consumers. The shift can be supported through the upscaling of insect production facilities will likely promote competitiveness within the market an inevitable drive down the price.

As can be noted throughout the research assignment, the potential for edible insects in reducing micronutrient deficiencies in South Africa does exist, however potential recommendations can be made to assist the process. Regarding the micronutrient deficiency statistics, possible recommendations would include focusing on collecting micronutrient data on a national level that is representative of the South African adult population. It is only possible to truly assess the current micronutrient deficiency status and to effectively plan and implement future strategies, if the available statistics are representative of the entire South African adult population. Regarding the edible insects’ nutrient content, the recommendation would be to specifically focus on conducting research and determination of edible insects’ nutritional content in South Africa. This would standardise the external factors to which the insect species are exposed to and will provide less disparity in the results obtained. Researchers will then be able to optimally assess which insect species have the most potential in acting as complimentary or supplementary food source. The aforementioned will

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also be applicable to the assessing the protein determination method of choice of edible insects. In controlling the external factors, for instance through intensive production facilities and in determining each insect specie with a variety of methods, the uniformity of the results will increase.

The field of entomophagy is full of potential, especially from a nutritional viewpoint in South Africa. Although gaps and uncertainty still exist, the future in entomophagy research and potential in assisting food and nutrition security are immense. This research assignment can serve as a platform for continuous research and aid in determining the nutritional content of edible insects in South Africa. The standardised results obtained will pave the path forward to promote production and market growth and potentially contribute to reduce the most prevalent South African deficiencies.

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